The 35th International Specialized Symposium on Yeasts

21 - 25 October 2019
Antalya, Turkey

"Yeast Cornucopia: Yeast for health and wellbeing"

PROCEEDINGS BOOK

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CONGRESS

The 35th International Specialized Symposium on Yeasts

is jointly organized by

International Commission on Yeasts (ICY) and

Cukurova University, Faculty of Agriculture, Department of Food Engineering Turkey

21-25 October 2019
Antalya, Turkey

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The 35th International Specialized Symposium on Yeasts

ISSY 35 2019
21-25 OCTOBER 2019
ANTALYA TURKEY
Yeast Cornucopia: Yeast for Health and Wellbeing

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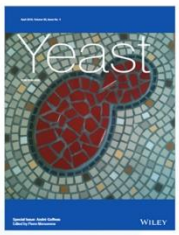

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The 35th International Specialized Symposium on Yeasts

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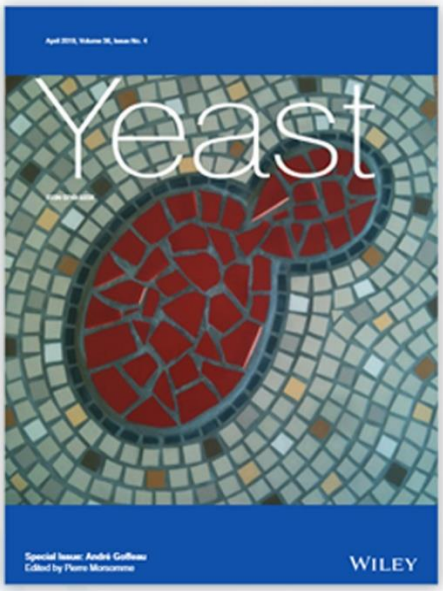

Dear Researchers,
A number of selected papers will be published as Full Text in the Yeast Journal Speacial Issue: "ISSY 35 Yeast Cornucopia: Yeast for Health and Wellbeing" after peer review.

Editor In Chief
Gianni Liti, France
Guest Editors for Special Issue:
Huseyin Erten, Turkey
Andriy Sibirny, Ukraine
Charles Abbas, USA

Additionally,
A number of selected papers will be published as Full Text in the Journal of Food, a publication of Association of Food Technology, Turkey - Speacial Issue after peer review process. Papers will be accepted in both languages; English and Turkish.

Editor
Kadir Halkman, Turkey
Guest Editors for Special Issue:
Huseyin Erten, Turkey
Turgut Cabaroglu, Turkey
Zerrin Erginkaya, Turkey

FROM THE ISSY 35 ORGANIZING COMMITTEE

Dear Friends and Colleagues,

On behalf of the Organizing Committee, I would like to thank you for your attendance of the 35th International Specialised Symposium on Yeasts. This is the first time the ISSY meeting is held in Turkey. The symposium is jointly organized by The International Commission on Yeasts (ICY) and the Department of Food Engineering, Faculty of Agriculture, Çukurova University.

Yeasts are important industrial microorganisms that have been used in numerous traditional as well as many new biotechnological applications. The theme of the 35th symposium is "Yeast Cornucopia: Yeast for health and wellbeing". The aim of the 35th ISSY is to provide an overview of the latest developments on yeast fermented foods and beverages, the production of ingredients and additives produced by yeasts, recent developments in yeast genetics and genomics as it relates to the yeast cultures used and taxonomic yeast characterization with emphasis on ecology and biodiversity, probiotic yeasts and the role of yeasts in improving health and wellbeing. Our hope by covering these topics is to expand new horizons and to broaden scientific knowledge in several of these areas.

The ISSY 35th symposium will have 1 plenary lecture, 8 keynote lectures and 54 oral talks and 78 poster presentations. The symposium has gathered around 200 distinguished senior, young and industrial scientists from over 44 countries that will present their latest research results while exploring the nearby beauties of Antalya as time permits. Some of our participants will visit the Olympus antique city ruins and the mountainous region surrounding the city as part of the social program that we hope they will enjoy. All abstracts, presentation slides, photographs and video recordings with authors permission, will be published at the webpage of the congress after the event, so please keep following our official webpage www.issy35.com

I would like to express my personal gratitude for ICY and ISSY35 Organizing Committee for all of their hard work and to the Scientific Committee members for their great efforts in evaluating many of the abstracts.

I am especially grateful to Turkish and International sponsors for their generous financial support. I also would like to thank to all participants, session chairs, speakers and Leon Congress who helped to turn the congress into a reality.

Welcome and thank you for attending ISSY35 and Turkey.

Prof. Huseyin Erten, Ph.D.

Chair of Organizing Committee of ISSY35

Head of Department,

Department of Food Engineering, Faculty of Agriculture,

Çukurova University, Adana, Turkey

FROM THE INTERNATIONAL COMMISSION ON YEASTS (ICY)

Dear Colleagues (ISSY35 Attendees),

On behalf of the International Commission on Yeasts (ICY), I welcome all of you to the International Specialized Symposium on Yeasts (ISSY35) held in Antalya, Turkey. This symposium is organized by Prof. Hüseyin Erten (Department of Food Engineering, Faculty of Agriculture, Cukurova University, Turkey), who is a great scientist in the field of food science and engineering in yeasts and lactic acid bacteria, and his wonderful team under the auspices of ICY. I'd like to express million thanks to his excellent job.

The subtitle of this symposium is "Yeast Cornucopia: Yeast for Health and Wellbeing", which covers a wide range of 'yeast science and technology' in fermented foods and beverages, probiotics, health, taxonomy, ecology, biodiversity, genetics and genomics, and as sources of ingredients and additives.

I wish all of you a productive meeting in Antalya, which is the eighth-most populous city in Turkey with over one million people, located on Mediterranean coast bordered by the Taurus Mountains. You can enjoy not only yeast science and technology but also a rich natural environment and historical/cultural tradition of Antalya and Turkey.

ICY <https://www.iums.org/index.php/home-icy> has been established in Bratislava in 1966 as the Council for Yeast Research, composed of prominent specialists in the field of yeasts. In 1971 the Council was transferred into the International Association of Microbiological Societies (IAMS), now International Union of Microbiological Societies (IUMS). ICY is now a commission under the Division of Mycology and Eukaryotic Microbiology. The general objectives of ICY are to establish an effective liaison between persons and organizations concerned with yeast investigations, and between them and the practical users of results of investigations including yeast culture collections. Every four years a General Symposium (International Congress on Yeasts; ICY) and each year in the meantime a Specialized Symposium (International Specialized Symposium on Yeasts; ISSY) will be held.

Welcome and thank you for coming to ISSY35 !!

Prof. Hiroshi Takagi, Ph.D.

Chair of ICY

Nara Institute of Science and Technology (NAIST), Japan

CONTENTS

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The 35th International Specialized Symposium on Yeasts

21 - 25 October 2019
Antalya, Turkey

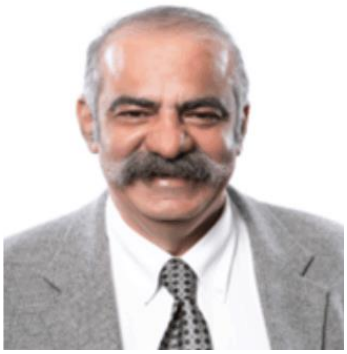
"Yeast Cornucopia: Yeast for health and wellbeing"

INVITED SPEAKERS

Charles A. Abbas, Ph.D., USA

Founder and CTO IBiocat Inc

University of Illinois



Charles received a B.S. in Microbiology from the U of Minnesota, an M.S. in Microbiology from the U. of Montana and completed a Ph.D. in Microbiology and Cell Science at the U. of Florida (Gainesville). He has over 30 years of experience in industrial biotechnology first working at Difco R & D in Ann Arbor, MI as a senior scientist, and as a group leader, manager and until recently the Director of Yeast and Renewables Research at Archer Daniels Midland (ADM) in Decatur, IL. Since retiring from ADM, Charles established a start up, IBiocat Inc, at the U of Illinois Research Park at Enterprise Works Incubator facility. IBiocat Inc focuses on the development, validaton, optimization and scale up of advanced biorefinery solutions in industrial biotechnology.

Dr. Abbas is the author of over 100 abstracts, scientific articles, book chapters, review articles, patents and patent applications. In April of 2016, Charles was awarded the Charles D. Scott for his contributions to the use of biotechnology for the production of fuels and chemicals. In June 2016, he was also awarded the BBI FEW Award of Excellence for his contributions to fuel ethanol. The Dr. Abbas is considered a leading expert in yeast, large-scale industrial fermentations, and biorefining. Charles is credited for proposing the widely used biorefinery term in early 1990s to illustrate the bioprocessing of commodity crops to high value added products. His areas of expertise include: industrial biotechnology; fungal chemistry and biochemistry; biomass conversion; advanced biofuels and chemicals; bioprocessing of commodity crops and their residues to produce high value-added products; development of yeast strains for industrial fermentations; large-scale fermentation production of ethanol, polymers, amino acids, enzymes, vitamins, carotenoids and organic acids using bacterial, fungal, and algal systems.

Pietro Buzzini, Ph.D., Italy

University of Perugia

Full Professor of Agricultural, Food and Environmental Microbiology, University of Perugia, Italy. Scientific Coordinator of the Microbiology Division, Department of Agricultural, Food, and Environmental Sciences, University of Perugia, Italy

Director of the Industrial Yeasts Collection DBVPG (www.dbvpg.unipg.it), affiliated to the European Culture Collection Organization and to the World Federation of Culture Collections.

Director of the Inter-University Research Center on Environment and Pollution “CIRIAF” (www.ciriaf.it), Universities of Perugia, Roma Tre, Florence, Pisa, L'Aquila, Rome "La Sapienza" and Polytechnic of Bari, Italy.

Delegate of the Chancellor of the University of Perugia (Italy) for the topic “green chemistry”.

Expertise: study of diversity, ecology, physiology of environmental yeasts, with special emphasis for cold-adapted (psychrophilic and psychrotolerant) yeasts in worldwide polar and non-polar cold habitats; study of biotechnological exploitation of cold adapted yeasts.

PI of some research projects on diversity, ecology, physiology and biotechnological significance of environmental yeasts granted by EU and Italian Institutions.

Co-author of over 220 abstracts, scientific papers, book chapters and reviews, and 1 EU patent. Co-editor of the books “Cold-Adapted Yeasts. Biodiversity, Adaptation Strategies and Biotechnological Significance", Springer, 2014; “Yeasts in Natural Ecosystems: Ecology”, Springer, 2017; “Yeasts in Natural Ecosystems: Diversity”, Springer, 2017.

The yeast species *Hyphopichia buzzinii* was named in his honor in recognition of his contribution to yeast research.

Associate Editor of Annals of Microbiology, Springer, since 2015.

Commissioner for Italy in the International Commission on Yeasts (www.iums.org/index.php/87-icy/138-international-commission-on-yeasts) since 2009.

Chair of the 32nd International Specialized Symposium on Yeasts (ISSY32), Perugia, Italy, 13-17 September 2015. Member of Scientific Committees/Advisory Boards session chair and invited keynote speaker in International Congresses.

He is also consultant for some “biotech” Companies.

Patrick Fickers, Ph.D., Belgium

University of Liège

Patrick Fickers has completed a Ph.D. in Biochemistry from University of Liège (Belgium) and Institut National Agronomic (Paris-France). After a postdoc at Polytech'Lille (France), he joined in 2005 the Centre of Protein Engineering (Liège, Belgium) as a FNRS fellow. Form 2009 until 2014, he was an Associated Professor at Université libre de Bruxelles and the head of the Biotechnology and Bioprocess Unit. Since 2015, he is a Professor at Gembloux Agro BioTech, University of Liège, in the Microbial Processes and Interaction (TERRA Teaching and Research Centre). His researches focus on the development of yeast cell factories (*Y. lipolytica* and *P. pastoris*) by metabolic engineering/synthetic biology and on process development in bioreactor for the production of value added chemicals.

Diethard Mattanovich, Ph.D., Austria

University of Natural Resources and Life Sciences (BOKU)

Dr. Mattanovich is Professor of Microbial Cell Design at the University of Natural Resources and Life Sciences, Vienna, Austria. His research interests focus on systems biology and metabolic engineering for the development of production processes for biochemicals and recombinant proteins, with a strong emphasis on yeast cell factories. Dr. Mattanovich holds a PhD in Biotechnology. He is Vice President of the European Federation of Biotechnology and serves as an Associate Editor of Microbial Cell Factories and Bioprocess and Biosystems Engineering.

Markus Ralser, Ph.D., UK

University of Cambridge

Markus Ralser (Einstein Professor of Biochemistry), is a Group Leader at the Francis Crick Institute (London), and since recently, Head of Division & Professor of Biochemistry, at the Charité, the Medical faculty in Berlin. Born in Italy, Markus studied Genetics and Molecular Biology in Salzburg (Austria), and completed a PhD studying Neurodegenerative disorders at the Max Planck Institute for Molecular Genetics (Germany).

After being trained in mass spectrometry at VU Amsterdam (The Netherlands), he started a Junior group at the Max Planck Institute for Molecular Genetics, before his lab moved to the University of Cambridge (to which Markus was affiliated until he accepted his Professorship in 2018). The Ralser lab is known for several fundamental discoveries, that have improved our understanding on how cells can coordinate hundreds of biochemical reactions assembled in the metabolic network. In particular, results obtained in the Ralser lab have provided key insights on how central carbon metabolism emerged in early life forms (driving the structure of modern metabolism), how reactions can co-occur within a cell despite competing for chemistry, and how yeast and cancer cells reconfigure metabolism rapidly to be protected against oxidative stress.

Recently, the Ralser lab has become known for the completion of a genome-scale functional-metabolomic analysis in a eukaryote, in which metabolomes were determined for 5,000 yeast strains that represent the non-essential yeast genome. This work has shown the scale of metabolic regulation necessary in the eukaryotic cell, that involves at least 1/3rd of the genome. The Ralser lab is/has been the recipient of prestigious grants by The Crick, the Wellcome Trust, the MRC, the ERC, EMBO, the BMBF, the Max Planck Society and the BBSRC. Markus Ralser has received several awards, in particular, the Wellcome-Beit prize, the BioMedCentral Research Award, the Colworth Medal of the Biochemical Society, as well as the Sterling Medal of the Endocrinology Society.

Andriy Sibirny, Ph.D., Ukraine/Poland

Institute of Cell Biology, NAS of Ukraine

University of Rzeszow, Poland

Prof. Andriy Sibirny is interested in the mechanisms of yeast autophagy, including that of specific autophagic degradation of peroxisome (pexophagy). Several new genes have been described including ATG26, ATG28, ATG35, TRS1, GSS1 etc. on the model yeast organisms *Komagataella phaffii* (*Pichia pastoris*), *Yarrowia lipolytica*, *Ogataea polymorpha* and *Saccharomyces cerevisiae*. Additionally, Andriy studies the mechanisms of autophagic degradation of soluble cytosolic proteins.

In the field of yeast biotechnology, Andriy and colleagues have constructed:

Strains of *S. cerevisiae* with an elevated production of first generation ethanol and an anaerobic overproducer of glycerol; strains of thermotolerant methylotrophic yeast *O. polymorpha* capable of active production of ethanol from pentose sugar xylose and by-product glycerol; overproducers of glycerol synthesis in O. *polymorpha* overproducers; of riboflavin and flavin nucleotides in the flavinogenic yeast *Candida famata*; isolated strains of *O. polymorpha* and *Escherichia coli* that are able to produce recombinant proteins as hepatitis surface antigen, glucose oxidase, amylolytic and xylanolytic enzymes and anticancer protein arginine deaminase

Hiroshi Takagi, Ph.D., Japan

Nara Institute of Science and Technology

His background is involved in Applied Molecular Microbiology that covers basic studies in microbial science and practical applications in biotechnology. To understand in depth microbial cells, we clarify and improve various functions and mechanisms of microorganisms. His best scenario is that novel findings and results of fundamental research can be applied to the construction of useful microorganisms (yeasts, bacteria), the production of valuable biomaterials (amino acids, enzymes) and the development of promising technologies to solve environmental and medical issues (bioethanol, neurodegenerative diseases).

Lene Jespersen, Ph.D., Denmark

University of Copenhagen

Lene Jespersen (LJ) is professor in Microbial Ecology and Food Fermentation at the Department of Food Science, University of Copenhagen (KU). LJ is educated at The Royal Veterinary and Agricultural University (MSc, Food Science 1989) and Industrial PhD Fellow (1994) based on collaboration between Alfred Jørgensen Laboratory Ltd., The Royal Veterinary and Agricultural University, and The Danish Academy of Technical Sciences. In her PhD, focus was on flow cytometric analysis for determination of single cell variability of brewing yeasts. In 1996 LJ joint KU and was in 2008 appointed as professor. Her research focuses on indigenous food products, food security, biotechnology, food microbiology, fermentation, microbial biodiversity as well as interactions within the human GI tract. Within these areas her main interests are within yeast taxonomy, yeast physiology and functionality as well as yeast interactions. LJ has headed several international, EU and national research projects as well as several research projects with the private sector. She has additionally worked with capacity building in developing countries for more than 25 years, primarily in Africa where her focus has been on up-grading the West African food sector focusing on food security and safety as well as education and private sector involvement. She has supervised more than 30 PhD students. Her dissemination output accounts >120 scientific publications and book chapters, >70 proceedings and >30 oral presentations at international and national scientific conferences.

Anne Christine Gschaedler Mathis, Ph.D., Mexico

The Center for Research and Assistance in Technology and Design of the State of Jalisco, (CIATEJ)

Dr. Anne Gschaedler is currently a senior researcher and director of the Zapopan Unit of the Jalisco State Technology and Design Research and Assistance Center (CIATEJ), located in Zapopan, Jalisco, Mexico. She has a PhD in Biotechnology and Food Industry (INPL, Nancy, France, 1994) and her research focuses on artisan fermentation processes (tequila, mezcal, cocoa), physiological studies of non-*Saccharomyces* yeasts and optimization of the process of fermentation. Inter-Institutional Postgraduate Professor in Science and Technology (PICYT) teaches molecular biology and biotechnology. Responsible for the training of several students (7 doctorate and 12 science teachers) and several research projects as well as in collaboration with the industry. She has 37 publications internationally and belongs to the National System of Researchers, level II.

SCIENTIFIC PROGRAM

21 October 2019, Monday - Adrasan Hall

08:00-09:00	Breakfast at Hotel
09:00-09:40	Opening Ceremony by Huseyin Erten, ISSY 35 Chair Mustafa Kibar, Cukurova University Rector Hiroshi Takagi, ICY Chair
09:40-10:20 Opening Lecture	Charles Abbas - University of Illinois & Ibiocat Inc., USA My Journey with Yeast: Exploiting Yeast Diversity for Industrial Applications
10:20-10:40	Coffee Break
Session 1: Yeasts in fermented foods and beverages Chairs: Charles Abbas & Huseyin Erten	
10:40-11:00	Francisco Carrau - University of the Republic, Uruguay Global Winemaking Application of *Hanseniaspora vineae* under Mixed Yeast Culture Conditions
11:00-11:20	María José Valera - University of the Republic, Uruguay ARO10 Genes Are Involved in Benzenoids Biosynthesis by Yeast During Wine Fermentation
11:20-11:40	Neil Jolly - ARC Infruitec-Nietvoorbij, South Africa Non-*Saccharomyces* Yeast in Wine – A South African Story
11:40-12:00	Agustin Aranda - University of Valencia-CSIC. Spain Activity of Nutrient Signalling Pathways of *Saccharomyces cerevisiae* During Winemaking
12:00-13:20	Lunch Break
Session 2: Yeasts in fermented foods and beverages Chairs: Hiroshi Takagi & Charoen Charoenchai	
13:20-14:00 Keynote Lecture	Hiroshi Takagi - Nara Institute of Science and Technology, Japan Improvement of Fermentation Ability and Product Quality in Industrial Yeast by "Functional Amino Acid Engineering"
14:00-14:20	Vivien Measday - University of British Columbia, Canada Whole Genome Sequencing of 75 *S. cerevisiae* Strains Isolated from Canadian Vineyards and Wineries
14:20-14:40	Angela Capece - University of Basilicata, Italy Interaction Between *Saccharomyces cerevisiae* and *Hanseniaspora uvarum* in Mixed Fermentation Affects Wine Characteristics
14:40-15:00	Nikola Gyurchev - University of Leicester, UK De Novo Yeast Hybrids with Enhanced Brewing Characteristics
15:00-15:20	Marc Serra Colomer - Carlsberg Research Laboratory, Denmark Unleashing the Brewing Potential of *Brettanomyces* Yeasts
15:20-15:40	Coffee Break
15:40-16:40	**Poster Session 1**

Session 3: Yeasts in fermented foods and beverages Chairs: Patrizia Romano & Vladimir Jiranek	
16:40-17:20 Keynote Lecture	Anne Christine Gschaedler Mathis - CIATEJ, Industrial Biotechnology, Mexico Non-*Saccharomyces* Yeasts in Food Fermentations and Beverages: Current Situation and Future Perspectives
17:20-17:40	Behnam Taidi - Ecole CentraleSupelec, France A New Process for Yeast Propagation for Champagne Production
17:40-18:00	Vasileios Englezos - University of Turin, Italy *Starmerella bacillaris* and *Saccharomyces cerevisiae* Interactions During Alcoholic and Malolactic Fermentations
18:00-18:20	Coffee Break
18:20-18:40	Vladimir Jiranek - University of Adelaide, Australia The Indigenous Microbiota of Australian Aboriginal and Torres Strait Islander Fermentations
18:40-19:00	Vanesa Postigo - Madrid Institute for Research and Rural Development in Food and Agriculture, Spain New Ingredients for The Fermentation of Beer: Non-*Saccharomyces* Yeasts from Wine
19:00-19:20	Kevin Verstrepen - VIB - KULeuven Center for Microbiology, Belgium Physiological Role and Industrial Opportunities of Yeast Aroma Production
19:20-20:30	Dinner (at hotel)
20:30-22:30	**Welcome Cocktail** (Vista Hall)

22 October 2019 Tuesday - Adrasan Hall

08:00-09:20	Breakfast at Hotel
09:20-09:40 Sponsor Speech	Sureyya Mert Selimoglu - Pakmaya (Pak Group of Companies), Turkey Industrial Production of Baker's Yeast – Current Trends and Challenges
Session 4: Probiotic Yeast Chairs: Lene Jespersen & Johan Thevelein	
09:40-10:20 Keynote Lecture	Lene Jespersen - University of Copenhagen, Denmark Beneficial Impact of Food-Borne and Probiotic Yeasts on Human Health – Traits That Determine Their Biological Functionality
10:20-10:40	Coffee Break
10:40-11:00	Johan Thevelein - VIB & KU Leuven, Belgium Unique Genetic Basis of The Distinct Antibiotic Potency of High Acetic Acid Production in the Probiotic Yeast *Saccharomyces cerevisiae* var. *boulardii*
11:00-11:20	Patricia Taillandier - Toulouse INP / LGC, France Kombucha Fermentation: Yeast Populations and Biological Activities
11:20-11:40	David Lapeña - Norwegian University of Life Sciences, Norway Production of Different Yeasts on Grown on Media Composed of Spruce Sugars and Protein Hydrolysates from Poultry By-Products
11:40-12:00	Ishtar Snoek - AB Biotek, Australia From Strain to Product
12:00-13:20	Lunch Break
Session 5: Yeast taxonomy, ecology, and biodiversity Chairs: Pietro Buzzini & Kyria Boundy-Mills	
13:20-14:00 Keynote Lecture	Pietro Buzzini - University of Perugia, Italy Polar and Non-Polar Cold Habitats: Little Explored Habitats for Fungal Life
14:00-14:20	Claire Brice - Stockholm University, Sweden The Frequency of Transgressive Segregants in Interspecific *Saccharomyces* Hybrids
14:20-14:40	Rameshwar Panditrao Avchar - MACS' Agharkar Research Institute, India Buffalo Rumen: A Potential Niche for Novel, Thermotolerant, and High Ethanol Producing Yeasts
14:40-15:00	Luís Ferraz - University of Milano Bicocca, Italy Membrane Engineering to Improve *Saccharomyces cerevisiae* Robustness Towards Formic Acid
15:00-15:20	Omer Simsek - University of Pamukkale, Turkey The Effect of Backslopping on the Yeast Diversity of Tarhana Fermentation
15:20-15:40	Coffee Break
15:40-16:40	**Poster Session 2**

Session 6: Yeast taxonomy, ecology, and biodiversity Chairs: Leda Mendonça-Hagler & Hyun Ah Kang		
16:40-17:00	Pooja Jayaprakash - University of Milano-Bicocca, Italy Adaptive Laboratory Evolution to Enhance Organic Acid Tolerance in *Kluyveromyces marxianus* on Residual Biomass	
17:00-17:20	Masako Takashima - Meiji Pharmaceutical University, Japan Fungal Diversity Based on Ortholog Analysis of Draft Genomes	
17:20-17:40	Mithat Kurban - Hacettepe University, Turkey DNA Barcoding of Yeast Strains from Anatolia	
17:40-18:00	Alexander Rapoport - University of Latvia, Latvia Intracellular Protective Reactions in Yeasts at Stress Conditions	
18:00-18:20	Seraphim Papanikolaou - Agricultural University of Athens, Greece Ethanol and Biomass Production by Newly Isolated Wild-Type Yeast Strains Cultivated on Glucose in Shake-Flask Experiments	
19:30-22:30	Dinner	ICY Commission Dinner

23 October 2019 Wednesday – Parallel Sessions

	Adrasan Hall	**Vista Hall**
08:00-09:20	Breakfast at Hotel	
09:20-09:40 Sponsor Speech	John Evans - AB Biotek, USA NGS for Industrial Yeast Development at AB Biotek	← *Please attend the Sponsor Speech*
	Session 7 **Yeast genetic and genomic** Chairs: Diethard Mattanovich & Mehmet Inan	**Session 10** **Yeast general** Chairs: Patricia Lappe-Oliveras & M. Evodia Setati
09:40-10:20 Keynote Lecture	Diethard Mattanovich - University of Natural Resources and Life Sciences, Austria Transcriptional Control Limits Central Carbon Metabolism of Crabtree Negative Yeasts: A Potential Role in Early Evolution of Fermentation?	← *Please attend the Keynote Lecture*
10:20-10:40	Coffee Break	
10:40-11:00	Zeynep Petek Çakar - Istanbul Technical University, Turkey Evolutionary Engineering and Molecular Characterization of Stress-Resistant Yeasts Using Systems Biology Tools	Hatice Aybuke Karaoğlan - Cumhuriyet University, Turkey Effect of Heat Pre-Treatment on Aminoacidic Profile in Fermentation of Synthetic Must Achieved by *Saccharomyces cerevisiae*
11:00-11:20	Matthias Sipiczki - University of Debrecen, Hungary Postzygotic Genome Evolution in *Saccharomyces* Interspecies Hybrids	Bilal Agirman - Cukurova University, Turkey Biocontrol Capability and Action Mechanisms of *Aureobasidium pullulans* and *Pichia guilliermondii* Against Blue and Green Moulds
11:20-11:40	Mehmet Inan - Izmir Biomedicine and Genome Center, Turkey Development of Synthetic *Pichia pastoris* Alcohol Dehydrogenase (ADH) Promoters	Cennet Pelin Boyaci Gunduz - Adana Alparslan Turkes Science and Technology University, Turkey Predominant Yeasts in the Sourdoughs Collected from Different Parts of Turkey
11:40-12:00	Emrah Nikerel - Yeditepe University, Turkey World of Small Molecules in Non-Conventional Yeasts	Nicola Francesca - Università degli studi di Palermo, Italy Fermented Honey and Manna Ash products: Novel Ecological Niches of Wine Yeasts
12:00-13:20	Lunch Break	
	Session 8 **Yeast genetic and genomic** Chairs: John Morrisey & Hana Sychrova	**Session 11** **Yeasts as sources of ingredients and additives** Chairs: Volkmar Passoth & Vladimir Mrša
13:20-14:00 Keynote Lecture	Patrick Fickers - University of Liège, Belgium Erythritol Metabolism in *Yarrowia lipolytica* and Engineering Tools Derived Thereof.	← *Please attend the Keynote Lecture*
14:00-14:20	Feng-Yan Bai - Chinese Academy of Sciences, China Adaptive Evolution of Sugar Metabolism Networks in Domesticated Lineages of *Saccharomyces cerevisiae*	Milan Čertík - Slovak University of Technology, Slovak Republic Metabolic Engineering of *Yarrowia lipolytica* for Production of Tailor-Made Lipid Compounds

	Adrasan Hall	Vista Hall
14:20-14:40	Petri-Jaan Lahtvee - University of Tartu, Estonia From Protein Translation to Fluxes – Quantitative Systems Biology of Yeast Stress Responses	Kyria Boundy-Mills - University of California Davis, USA Conversion of Sugars and Phenolic Compounds to Secreted Surfactants by *Rhodotorula* yeasts
14:40-15:00	Eduvan Bisschoff - University of the Free State, South Africa The Development of a Wide Range CRISPR-Cas9 Gene Editing System for Yeast.	Małgorzata Kus-Liśkiewicz - University of Rzeszow, Poland Yeast *Cornucopia* – Bioinspired Gold Nanoparticles Synthesis
15:00-15:20	Tiina Alamäe - University of Tartu, Estonia Maltase of *Blastobotrys adeninivorans* Displays Unusual Properties and Has Biotechnological Potential	Zbigniew Lazar - Wrocław University of Environmental and Life Sciences, Poland Biotechnological Applications of the Yeast *Yarrowia lipolytica*
15:20-15:40	Coffee Break	
	Session 9 **Yeast genetic and genomic** Chairs: Andriy Sibirny & Vivien Ruth Measday	**Session 12** **Yeasts as sources of ingredients and additives** Chairs: Patrick Fickers & Elena S. Naumova
15:40-16:20 Keynote Lecture	Andriy A. Sibirny - Institute of Cell Biology NAS of Ukraine, Ukraine Construction of the Advanced Producer of Riboflavin on Whey and Lignocellulose Hydrolyzates in the Flavinogenic Yeast *Candida famata*	← *Please attend the Keynote Lecture*
16:20-16:40	Liliane Barroso - University of Leicester, UK Insights into Phenotypic and Genetic Characteristics of the Spoilage Yeast *Zygosaccharomyces (para) bailii*	John Morrissey - University College Cork, Ireland Synthetic Strategies for Production of Aromatic Molecules in *Kluyveromyces marxianus*
16:40-17:00	Paola Branduardi - University of Milano Bicocca, Italy Improving Yeast Stress Tolerance by the Synthetic Shuffling of the Polya Binding Protein (Pab1) Domains	Volkmar Passoth - Swedish University of Agricultural Sciences, Sweden Biofuels, Feed and Food Production from Lignocellulose Using *Oleaginous* Yeasts
17:00-17:20	Justyna Ruchala - University of Rzeszow, Poland Construction of The Riboflavin-Overproducing Strain of the Yeast *Komagataella pastoris* Producing the Flavin Antibiotic Aminoriboflavin	Nazanin Bolghari - Pasteur Institute of Iran, Iran A Novel Chimeric Bioadhessive Protein Production in *Pichia pastoris* for Biomedical Applications
17:20-17:40	Jacobus Albertyn - University of the Free State, South Africa CRISPR-CAS9 Gene Editing Tools in Pathogenic and Non-Pathogenic Yeasts	← *Please attend to the Vista Hall for special event*
17:40-21:00	**LEON Congress Special Event** **"Knowledge Based Treasure Hunting"**	
21:00-23:40	Gala Dinner (Colors Restaurant)	

24 October 2019 Thursday, Vista Hall

08:00-09:20	Breakfast at Hotel	
Session 13: Yeasts in health Chairs: Markus Ralser & Feng-Yan Bai		
09:20-10:00 Keynote Lecture	Markus Ralser - Charité University Medicine, Germany Towards Explaining the Metabolome of a Yeast Cell	
10:00-10:20	Nitnipa Soontorngun - King Mongkut's University of Technology Thonburi, Thailand The Yeast Transporter Pdr5 Confers Resistance to the Antifungal Cytochalasin of *Xylaria* sp. BCC 1067	
10:20-10:40	Oluwasegun Kuloyo - University of the Free State, South Africa Arachidonic Acid Increases Expression of CDR1 in *C. albicans*, But Inhibits Efflux Activity	
10:40-11:00	Hana Sychrova - Institute of Physiology Czech Academy of Sciences, Czech Republic Trk Transporters Mediate Potassium Uptake and Contribute to Cell pH Homeostasis and Fitness of Pathogenic *Candida* Species	
11:00-11:40	Closing Ceremony	
11:40-12:10	Hotel Check-Outs	
12:10-13:30	Lunch Break	
13:30-18:30	Social Event Cable-car Trip to Olimpos Mountains and Ancient City Tour with Sunset View	12:10 Social Event Kemer Scuba Diving Tour (Lunch at the Yatch)

25 October 2019, Friday - Vista Hall

WORKSHOP

08:00-09:20	Breakfast at Hotel
09:20-10:40	Overview of Recombinant Protein Production in Yeast (*Pichia pastoris*)
10:40-11:00	Coffee Break
11:00-12:00	Strain Development (*Pichia pastoris*)
12:00-13:20	Lunch Break
13:20-13:45	Transfer to Akdeniz University
13:45-15:00	Fermentation Basics and Applications
15:00-17:30	Hands on Experience in the Lab
17:30-18:00	Transfer to Hotel

Workshop Instructor

Prof. Mehmet Inan (PhD)

Assistant Director

Technological Research Program

Izmir Biomedicine and Genome Center (IBG)

Izmir, Turkey

The 35th International Specialized Symposium on Yeasts

21 - 25 October 2019
Antalya, Turkey

"Yeast Cornucopia: Yeast for health and wellbeing"

ORAL PRESENTATIONS

Opening Lecture

My Journey with Yeast: Exploiting Yeast Diversity for Industrial Applications

ORAL PRESENTATION ID: 249

Charles Abbas

Founder & CTO, Ibiocat, Incorporated
University of Illinois Urbana-Champaign, USA

The contribution of microorganisms to the health and wellbeing of mankind goes back several millennia. Microorganisms have long been used to enhance food and beverage flavors and to aid in preservation of grains, fruits, vegetables, meat, poultry and dairy products beyond harvest. Fungi, and, in particular, yeasts and molds, have been deployed for centuries in the industrial production of foods, beverages, and animal feed. Much of the early use of yeasts relied on inoculation from natural sources and then the use of routine sub-culturing of adapted strains, which continue to evolve and mutate; in the process, variants that change with changes in substrates and conditions are being selected. The development of controlled fermentation and the use of monocultures dates back to the late 18th century with the development of modern brewing techniques and the industrial production of beer and other fermented alcoholic and non-alcoholic beverages. Since then, great progress has been made in yeast strain improvement using simple genetic tools similar to the approaches used for animal and plant hybrid development. These tools paved the way for greater understanding of the role that genetics plays and led to further improvement in fermentation strains through the use of mutagenesis followed by extensive selection. These approaches have been coupled with improvement in modern fermentation technology that started with the industrial production of penicillin during World War II. The intersection of strain development with chemical engineering further expanded the range of fermentation products to chemicals, biofuels, pharmaceuticals, and nutraceuticals. Recent developments in the industrialization of yeast have used genetic engineering for harnessing and genomic mining of genes from a wide range of sources. In the past two decades, continued development of genetic engineering has widely expanded the product range, thereby heralding the arrival of a new age of biotechnology – called The Era of the Biofoundry -- where industrial biomanufacturing is gaining momentum.

As an industrial scientist, over the past four decades I have witnessed first-hand the rapid pace of development in yeast biotechnology and its use at modern state-of-the-art biorefineries. From amino acids for use in animal feeds to beverages, from biofuels to biobased chemicals, from carotenoids to flavonoids**,** from nutraceuticals to pharmaceuticals, from polymers to probiotics, and from sweeteners to vitamins - these uses all demonstrate how the simple eukaryotic organisms we know as yeasts can be turned into “microfactories” that can help address a range of human needs, and replace many products that are derived from fossil fuels. At the heart of all this is the ability of science to deliver improvements in the manufacturing of biobased products by using natural abundant renewable sources while tapping into yeast biodiversity. These are examples of what science and biotechnology can deliver. These are exciting times but are we up to the challenges.

Keywords: Yeast Diversity

SESSION 1: Yeasts in Fermented Foods and Beverages

Global Winemaking Application of *Hanseniaspora vineae* Under Mixed Yeast Culture Conditions

ORAL PRESENTATION ID: 143

***Francisco Carrau*[1], *Karina Medina*[1] *María José Valera*[1], *Eduardo Dellacassa*[2], *Valentina Martin*[1], *Eduardo Boido*[1], *Gabriel Perez*[1], *Valentina Olivera*[1], *Remi Schneider*[3], *Albert Mas*[4], *Sergio Echeverrigaray*[5], *Laura Fariña*[1,2]**

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Hanseniaspora genus is the main yeast group isolated from grapes and musts. It includes ten wine species that have been associated in two clades defined as the fruit and fermentation adapted groups. We focused our interest on the application of *H. vineae*, in our opinion the main species of the fermentation clade. In this work, we studied winemaking potential of *H. vineae* in order to understand how to apply it, taking advantage at winemaking level of its capacity to produce wines with increased sensory complexity compared with *Saccharomyces cerevisiae*.

This species is evaluated in some practical aspects for its fermentation capacity at low temperature and at low assimilable nitrogen levels at laboratory scale and 225 liters oak barrels. Furthermore, it lacks of H2S production and have good SO_2 resistance. Other desirable effects were also studied, as its ability to increase flavor complexity with neutral grape varieties (such as Trebbiano, Macabeo or Semillon) and cell autolysis by flow cytometry analysis. Yeast-yeast interactions during fermentation were evaluated with different commercial *S. cerevisiae* strains in order to screen growth and fermentation synergistic capacities.

Results showed that *H. vineae* loss of membrane integrity might explain increase mouthfeel impact compared to *S. cerevisiae*. Protease and β-glucosidase activity in grape must or wine, and promotion of malolactic fermentation was confirmed. *H. vineae* presents slower fermentation rate, considered and advantage due to the decrease flavor losses and energy demand for cooling the process compared to *S. cerevisiae*. Contribution of *H. vineae* with some key aroma compounds such as benzenoids, sesquiterpenes, acetate esters, and decanoic acid was consistent compared to *S. cerevisiae* solo fermentation. These attributes were reflect in the sensory evaluation, where all of the fermentations performed in grape juice mediums with *H. vineae* were considered superior to those from *S. cerevisiae*. Moreover, its ability of being dried as an active formulation is discussed and will make possible to extend its global market application in winemaking

In conclusion, although *H. vineae* shows a great capacity to produce differentiated wines, some fine tuning adjustments are necessary to improve end of fermentation by a sequential inoculation of *S. cerevisiae* at the middle of the process. Nutrient management and mixed strain selection for this step should be carefully defined to complete total sugar depletion.

Keywords: *Hanseniaspora vineae*, wine aroma, active dry yeasts

Acknowledgements: We thank the National Agency for Innovation ANII and CSIC office of UdelaR.

ARO10 Genes Are Involved in Benzenoids Biosynthesis by Yeast During Wine Fermentation

ORAL PRESENTATION ID: 142

***María José Valera*[1], *Eduardo Boido*[1], *Eduardo Dellacassa*[2], *Gemma Beltran*[3], *María Jesús Torija*[3], *Albert Mas*[3], *Francisco Carrau*[1]**

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Benzenoids are compounds associated with positive flavour characteristics in wines, being most of them originated from grape metabolites. However, some yeasts are also able to synthesize them from aromatic amino acids. The complete metabolic route for the production of volatile benzenoids remains unknown, except in plants where the phenyl ammonia lyase pathway is well-known. *Hanseniaspora vineae* can produce benzenoids up to two orders of magnitude more than *Saccharomyces* species, so we proposed it as a model for studying benzenoids biosynthesis pathways in yeast species. According to their genomes, several enzymes have been proposed to be involved in a mandelate pathway similar to that described for some prokaryote cells. Among them, in *H. vineae*, ARO10 homologous genes present predicted domains similar to *Pseudomonas* benzoyl formate decarboxylase protein. This enzyme catalyzes the decarboxylation of benzoyl formate into benzaldehyde at the end of the mandelate pathway in benzyl alcohol formation.

Two homologous genes of ARO10 were found in the sequenced *H. vineae* strains. For laboratory and industrial *S. cerevisiae* strains, the ARO10 single copy gene codifies for phenyl pyruvate decarboxylase and is involved in the first specific step of the Ehrlich pathway. In this study, nine *H. vineae* strains were analysed to detect the presence, and homology percentage, of ARO10 sequences by PCR using specific primers designed for this species. Also, the copy number of the genes was calculated by quantitative PCR. To verify the relation of ARO10 with the production of benzyl alcohol, a deleted mutant in ARO10 gene of *S. cerevisiae* was used to check the production of this alcohol during fermentation.

The results showed the presence of homologous sequences of both genes ARO10 in seven strains of *H. vineae*. Some of them presented more than two copies of ARO10 that, in some cases, correlated with higher benzyl alcohol production in *H. vineae*. On the other hand, *S. cerevisiae* mutated in ARO10 was unable to produce neither benzyl alcohol nor 4- hydroxybenzaldehyde.

In conclusion, ARO10 homologous genes are involved in benzenoids biosynthesis pathway in yeast and might have a key function in the mandelate pathway as a benzoyl formate decarboxylase regarding to sequence homology. This gene might be also key on the mandelate pathway using tyrosine as the precursors of the 4-hidroxybenzaldehyde formation. Further research of the mandelate pathway role in yeast is in progress in *H. vineae*, a good model for eukaryotic cells.

Keywords: *Hanseniaspora*, benzenoids metabolism, wine aroma.

Non-*Saccharomyces* Yeast in Wine – A South African Story

ORAL PRESENTATION ID: 141

***Neil Jolly*, Heinrich du Plessis**

ARC Infruitec-Nietvoorbij, Stellenbosch, South Africa

Wine microbiologists have long been interested in non-*Saccharomyces* yeasts and their role in wine. During the 1960's much attention was given to these yeasts when isolated from stuck fermentations and spoiled wine. However, even then it was recognised that some of these yeasts formed metabolites not necessarily detrimental to wine quality. In the mid-1990's there was a renewed interest in non-*Saccharomyces* yeast, but this time specifically for their beneficial role in wine production. The aims of the research were to enhance wine quality and to diversify wine styles by using these yeasts naturally found in grape musts.

An initial survey of yeast in vineyards and in grape musts, showed the presence of four dominant species, *Metschnikowia pulcherrima, Torulaspora delbrueckii, Candida zemplinina* and *Hanseniaspora uvarum.* These yeasts were trialled in laboratory-, small- and pilot-scale wine trials as co-inoculants with *S. cerevisiae* in various white and red grape fermentations.

Specific non-*Saccharomyces* yeast and grape variety combinations were shown to improve wine quality. *C. zemplinina,* could improve fermentation efficiency in grape musts with initial high levels of fructose, thereby lessening the danger of stuck fermentations. In addition, the use of non-*Saccharomyces* yeasts always led to a slight reduction in alcohol levels, a property that could be used in combination with viticulture and other oenological practices for lowering alcohols in wines to consumer acceptable levels. Counter productively, interactions between non-*Saccharomyces, S. cerevisiae* and lactic acid bacteria can negate the positive effect of the non-*Saccharomyces* yeast, so the choice of non-*Saccharomyces, S. cerevisiae* and lactic acid bacteria combination is important.

For yeast manufacturers, producing non-*Saccharomyces* yeast is a challenge, making them an expensive ingredient for wine production. This, together with sometimes insufficient implantation during fermentation, has hampered the wide-scale acceptance of these yeasts. However, the positive benefits arising from replicating a spontaneous fermentation by using non-*Saccharomyces* yeast in a controlled manner will ensure that non-*Saccharomyces* yeast will continue to take their rightful role in wine production.

Keywords: Non-*Saccharomyces*, Wine

Acknowledgements: Agricultural Research Council (ARC Infruitec-Nietvoorbij) National Research Foundation

Activity of Nutrient Signalling Pathways of *Saccharomyces cerevisiae* During Winemaking

ORAL PRESENTATION ID: 130

***Agustín Aranda*, Beatriz Vallejo, Emilia Matallana**

Institute for Integrative Systems Biology. University of Valencia/CSIC (Spain)

Grape juice fermentation is carried out by wine strains of *Saccharomyces*, mainly *S. cerevisiae*. During this process, the crosstalk between nutrients signalling pathways are of great interest to ensure growth and an efficient metabolic transformation of the substrate. cAMP-PKA and TORC1 pathways promote growth and cell proliferation when glucose and nitrogen respectively are plenty. Wine yeast were found to differ from laboratory strains regarding tolerance to chemical inhibitors of several of these pathways.

As nitrogen starvation is the nutritional limiting factor during winemaking, we studied TORC1 targets by western blot analysis. Phosphorylation of Rps6p and Par32p (both of them readouts of TORC1) indicate that this pathway seems to be active only in the first hours of winemaking, even when growth has not ceased. Snf1, is the AMPK kinase which is usually activated when glucose is consumed in order to use alternative carbon sources. It is surprisingly phosphorylated and active during early winemaking, so additional stress conditions at the beginning of fermentation may give rise to a certain level of kinase activation, despite the high abundance of sugars in the grape must. Gln3p, a nitrogen catabolic repression (NCR) transcription factor, is both a TORC1 and Snf1 target. NCR system represses the use of poor nitrogen sources when optimal sources are present. Phosphorylation level of Gln3p indicate that NCR system is activated from one day of fermentation, even before nitrogen is consumed, probably due to nitrogen storage. In this condition other nutrient signalling pathways, more sensitive to carbon sources, could be active to promote growth. PKA shows a complex pattern of activation, with both early and late targets during winemaking, suggesting that it may be overseeing other pathway's activities.

The coordination between nutrient signalling pathways integrate different inputs, like nitrogen shortage and high sugar, to respond to the specifically environmental conditions during the alcoholic fermentation of the grape must.

Keywords: Nutrient signalling pathways, winemaking, TORC1, Snf1, NCR, PKA

Acknowledgements: This work has been funded by a grant from the Spanish Ministry of Economy and 591 Competiveness MINECO (AGL2017-83254-R). B.V. is a F.P.U. fellow.

SESSION 2: Yeasts in Fermented Foods and Beverages

Improvement of Fermentation Ability and Product Quality in Industrial Yeast by "Functional Amino Acid Engineering"

ORAL PRESENTATION ID: 126

Hiroshi Takagi

Nara Institute of Science and Technology, Nara, Japan

There are two major purposes for breeding of industrial yeast: improvement of fermentation ability with enhanced tolerance to environmental stresses during fermentation processes; and diversity of product taste and flavor with modified metabolic pathways. Yeast cells are subjected to various environmental stresses during fermentation, including high ethanol concentration, extreme temperatures, freezing, drying, and high osmotic pressure. Such stresses induce protein denaturation and generation of reactive oxygen species (ROS), inhibiting growth or causing cell death. Severe stress conditions can reduce the fermentation efficiency of yeast. Thus, in terms of industrial application, stress tolerance is key for yeast cells. Metabolites produced by yeast during fermentation, including amino acids, organic acids, fatty acids, saccharides, alcohol, and ester, influence the taste or flavor of fermented products.

Amino acid is one of the key factors affecting yeast fermentation. All L-amino acids (except L-lysine, L-histidine, and L-cysteine) can support yeast cell growth as the sole nitrogen source, although there are differences between strains. In yeast, amino acid metabolism and its regulatory mechanisms vary under different growth environments by regulating anabolic and catabolic processes, including uptake and export, and the metabolic styles form a complicated and robust network. There is also crosstalk with various metabolic pathways, products and signal molecules. The elucidation of metabolic regulatory mechanisms and physiological roles is important fundamental research for understanding life phenomenon. Very few studies, however, have considered the effects of amino acids during fermentation. It is thought that breeding yeasts with various amino acid composition profiles could expand the diversity of product tastes. In terms of industrial application, the control of amino acid composition and content is expected to contribute to an improvement in productivity, and to add to the value of fermented foods, alcoholic beverages, bioethanol, and other valuable compounds. The yeast *Saccharomyces cerevisiae* is considered reliable and safe in food production, and thus the development of novel strains that overproduce amino acids would represent a significant contribution to food-related industries.

To improve fermentation ability and product quality in industrial yeast, I will introduce a new breeding technology called "Functional Amino Acid Engineering", focused on the metabolic regulations and physiological roles of amino acids found in yeast (*Biosci. Biotech. Biochem.*, doi: 10.1080/09168451.2019.1576500, in press).

Keywords: *Saccharomyces cerevisiae*, Amino acid, Metabolic regulation, Physiological function

Whole Genome Sequencing of 75 *S. cerevisiae* Strains Isolated from Canadian Vineyards and Wineries

ORAL PRESENTATION ID: 201

***Vivien Measday*[1], *Sneha Ralli*[2,3], *Jay Martiniuk*[1], *Jonah Hamilton*[1], *Kishori M Konwar*[4] *Aria Hahn*[4]**

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Yeasts, namely species of the genus *Saccharomyces*, found in vineyards and wineries are increasingly recognized as an important contributor to a wine's regional character or terroir. Based on previous genotyping analyses, we have identified unique sub-populations of *S. cerevisiae* strains in the Okanagan Valley, a Canadian winemaking region in the province of British Columbia. To fully elucidate the genomic structure of our *S. cerevisiae* populations, we have performed whole genome sequencing (WGS) of 75 *S. cerevisiae* strains isolated from vineyards and wineries in the Okanagan valley wine region.

PCR-free genomic libraries were constructed for each *S. cerevisiae* genome and sequenced using an Illumina HiSeq X paired-end approach with 162-fold mean coverage. Single nucleotide polymorphisms (SNPs) and insertions/deletions were predicted using GATK. Phylogenetic analyses of all 75 strains were done using biallelic SNPs and over 1,000 published *S. cerevisiae* genomes. Reads not mapping to S288C were assembled de novo and open reading frames (ORFs) were predicted. These ORFs were aligned to two published *S. cerevisiae* pan-genomes to identify industrially relevant genes while remaining ORFs were annotated using RefSeq. Ten commercial wine strains and 10 Okanagan strains were assembled and scaffolded using S288C. Variations in genomic arrangement from the reference were verified with unassembled reads.

On average, 97.7% of reads mapped to the S288C reference genome. Based on preliminary analyses of copy number variation (CNV) of genes in S288C, there are three subpopulations within the 75 genomes. Detailed phylogenetic analyses comparing the Okanagan strains to global *S. cerevisiae* strains profiled in Borneman et al., 2016 G3, 6(4):957 and Peter et al., 2018 Nature, 556(7701):339 will verify these results. Collectively, the 75 Canadian *S. cerevisiae* wine strains contain over 125 ORFs not present in the S288C genome. Some of the non-S288C ORFs are 100% identical to genes identified in *S. cerevisiae* strains isolated from other industrial processes (bioethanol, sake) or clinical samples, while some remained unannotated. Gene CNV is prominent at the subtelomere regions and include hexose transporter and alcohol dehydrogenase genes. Following joint genotyping of all 75 strains, 65,446 SNPs were identified and are being analyzed further.

We have carried out the first WGS of Canadian *S. cerevisiae* strains and added 75 strains to the list of ~450 winery, vineyard and commercial wine *S. cerevisiae* strains that have been sequenced. Our strains contain new ORFs, unique CNV, a high number of SNPs and genome rearrangements.

Keywords: *S. cerevisiae*, Wine, Vineyards, Whole genome sequencing

Acknowledgements: Investment Agriculture Foundation of British Columbia, Okanagan Crush Pad Winery, Stoneboat Vineyards Winery, Tinhorn Creek Vineyards, Les Dames d'Escoffier, British Columbia Chapter.

Interaction Between *Saccharomyces cerevisiae* and *Hanseniaspora uvarum* in Mixed Fermentation Affects Wine Characteristics

ORAL PRESENTATION ID: 132

***Angela Capece*[1], *Angela Pietrafesa*[1], *Marina Bely*[2], *Patrizia Romano*[1]**

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Although the presence of *Hanseniaspora uvarum* is limited to the early fermentation phases, it's known that the metabolism of *H. uvarum* affects significantly the final quality of wine, also when *S. cerevisiae* is added as starter culture. Furthermore, the actual trend in winemaking is addressed to revalue the role of non-*Saccharomyces* yeasts for increasing the sensory complexity of wines, promoting the use of starter cultures composed by *S. cerevisiae* and non-*Saccharomyces* strains. However, the use of mixed starters needs to a better understanding of the interaction mechanisms between these species during alcoholic fermentation. The aim of this study is to evaluate the influence of mixed starter cultures, composed by combination of different *S. cerevisiae* and *H. uvarum* strains, on wine characteristics.

Wild strains (three *H. uvarum* and two *S. cerevisiae*), previously selected, were tested during mixed fermentations in natural red grape must. The fermentation course was monitored by evaluating CO_2 evolution and the experimental wines were analyzed for content of compounds affecting wine aroma, antioxidant power and total polyphenols content. One selected mixed starter was tested in fermentations in the bioreactor with and without separated compartments, by monitoring fermentative process, yeast cells evolution and analysis of aromatic fraction of experimental wines.

The analysis of experimental wines from the different mixed starters tested revealed that the presence of apiculate yeasts affects wine characteristics, although the results were variable in function of yeast strains included in the mixed starters. As regards the fermentation with double-compartment bioreactor, the decrease of viable cell population of *H. uvarum* was higher when *H. uvarum* and *S. cerevisiae* strains were not physically separated compared to the fermentation performed with physical separation between the two species. Furthermore, the analysis of experimental wines showed that inoculum modality affects the content of secondary compounds, such as esters, which were higher in wines obtained from non-separated inoculum than experimental wines obtained by inoculating the two species separately.

These results confirmed the high biodiversity among wild strains and the existence of interaction mechanisms due cell to cell contact between *H. uvarum* and *S. cerevisiae*, which affect both cell viability and metabolic behavior.

Keywords: *H. uvarum*/*S. cerevisiae*, Yeast interaction

Acknowledgements: This work was supported by the project PSR Regione Basilicata 2014-2020 Sottomisura 16.1 GO Vite&Vino PROduttività e Sostenibilità in vITIvinicoltura - (PROSIT)-N. 54250365779

De Novo Yeast Hybrids with Enhanced Brewing Characteristics

ORAL PRESENTATION ID: 182

***Nikola Yuliyanov Gyurchev*[1], *Niels Kuijpers*[2], *Elke Nevoigt*[3], *Edward J. Louis*[1]**

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Lager beer produced with the yeast *Saccharomyces pastorianus* predominates the beer market. Therefore, research related to this hybrid yeast is of high interest. One parent of the hybrid is long known to be *Saccharomyces cerevisiae*. In the last decade, the other parent was discovered to be *Saccharomyces eubayanus*. The genetics behind phenotypes relevant to brewers is of great interest; however, research has been hindered by the sterility of the hybrid and its complex aneuploid genome. Now that the genome origins of the hybrid have been revealed, generating de novo hybrids was a logical next step and several examples of de novo *S. pastorianus* strains have been reported. The biodiversity within the *Saccharomyces* genus inspired researchers to exploit this diversity in an even broader way and use further unconventional species for the construction of hybrids with novel or enhanced brewing characteristics. While some of these potential hybrid combinations have been previously reported, several combinations are yet to be explored.

The aim of the current project is to generate genetically diverse de novo brewing yeast hybrids with improved process characteristic or with interesting flavour profiles. To this end, nearly 200 strains from the 8 different species of the *Saccharomyces* genus collected from various environments were high-throughput pre-screened on relevant properties such as growth on different wort sugars and ethanol tolerance at 12°C. In order to promote diversity, hybrids were constructed not only between *S. cerevisiae* and *S. eubayanus*, but also with more combinations of candidates exhibiting desirable phenotypes. To further diversify phenotypes, the obtained hybrids can be mated. This is, however, a challenge since hybrids are sterile. In this project we try to overcome this sterility via an intermediate tetraploid as previously described. This will enable genetic analysis on their progenies by QTL mapping to discover valuable correlations between genotype and phenotype in those complex genomes.

Keywords: Lager, Yeast hybrids, Biodiversity

Unleashing the Brewing Potential of *Brettanomyces* Yeasts

ORAL PRESENTATION ID: 119

***Marc Serra Colomer*[1,2], *Anna Chailyian*[1], *Ross T. Fennessy*[1] , *Kim Olsson*[1], *Natalia Solodovnikova*[1], *Birgitte Funch*[1], *Jochen Forster*[1]**

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Brettanomyces yeast are creating attraction in many sectors of the biotechnological industry. Exclusive properties such as high ethanol production, efficient sugar utilization, pH tolerance or release of unique flavors have brought this yeast species into focus of the brewing industry.

In this project, we sequenced the whole genome of a range of different *Brettanomyces* isolates from all over the world. The collection comprises mainly isolates from breweries and wineries, and selected isolates from bioethanol plants and sodas. We analyzed the genomic set-up of the *Brettanomyces* collection, and we checked its potential for brewing. High-throughput micro-scale beer fermentations were performed followed by analysis of flavor metabolites and in-vitro assays.

Different genomic patterns were identified that lead to specific brewing-relevant phenotypes. We revealed different sugar assimilation preferences related to the substrate of isolation of the strain. We identified a set of strains producing intensive flavors. In addition, we identified unique *Brettanomyces* strains with high production of esters.

Brettanomyces yeasts provide novel opportunities for the beer. The yeast exhibits strong strain variability, genotypically and phenotypically. The selection of the correct strain with the right properties is crucial to achieve the desired results in the final product.

Keywords: Yeast diversity, Genomics, Beer flavor, High-throughput

SESSION 3: Yeasts in Fermented Foods and Beverages

Non-*Saccharomyces* Yeasts in Food Fermentations and Beverages: Current Situation and Future Perspectives.

ORAL PRESENTATION ID: 248

Anne Christine Gschaedler Mathis

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In the past considered as undesired or spoilage yeasts now the non-*Saccharomyces* play an increasing role in enhancing the composition and aroma profile of wine, alcoholic beverages and others fermented foods.

In the case of fermentations to obtain alcoholic beverages, more than 40 species of yeasts have been reported, belonging to the following genera: *Aureobasidium*, *Brettanomyces*, *Candida*, *Clavispora*, *Cryptococcus*, *Debaryomyces*, *Hanseniaspora*, *Hanseluna*, *Issatchenkia*, *Kluyveromyces*, *Lachancea*, *Metschnikowia*, *Meyerozyma*, *Pichia*, *Rhodotorula*, *Saccharomycodes*, *Starmerella*, *Wickerhamomyces*, *Torulaspora* and *Zygosaccharomyces*. Some strains of these species have been selected and are already available in the market as starters based on non-*Saccharomyces*. It is the case of *Lachancea thermotolerans*, *Metschnikowia pulcherrima*, *Pichia kluyveri*, *Torulaspora delbrueckii* and *Zygosaccharomyces pombe* for applications in wine fermentations. In the case of beer there is also a great search for new yeasts that give the final product some specific characteristics (mainly related to aroma profile).

In other fermented foods such as dough fermentation, non-*Saccharomyces* intervention has been reported too. Some species such as *Kazachstania gamospora*, *Wickerhamomyces subpelliculosus* and *Torulaspora delbrueckii* demonstrate great potential in the production of CO_2 as well as aromas.

Another interesting and new application of non-*Saccharomyces* is as a tool for reducing the potential ethanol content in wines. Other reports (in wine fermentation) mention the release of varietal aromas, such as terpenes and thiol thanks to the generation of specific enzymes. Another new application in food is its possible prebiotic effect.

Now to be able to use these non-*Saccharomyces* strains commercially, a great challenge is their conservation and the obtaining of practical products for use in industry. The use of active dehydrated yeast (ADY) is common in the industry that employs *S. cerevisiae*. The big question is how these new strains of non-*Saccharomyces* resist these conservation processes, which represent a strong stress that has an important impact on their viability after the conservation stage. This topic has been poorly studied and the results available demonstrate a strong impact of classical conservation techniques on non-*Saccharomyces* yeasts. Possible strategies will be presented to ensure adequate conservation of these types of strains.

Keywords: Non-*Saccharomyces*, Food fermentation, Yeasts conservation

A New Process for Yeast Propagation for Champagne Production

ORAL PRESENTATION ID: 157

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The Crabtree effect is the preference of an organism for a fermentative metabolism in the presence of oxygen when the carbon substrate is in abundance. This phenomenon is noticeable in *Saccharomyces cerevisiae* and has been made use of for thousands of years for the production of alcoholic beverages under conditions of partial aerobie. The Crabtree effect also poses an industrial problem when wishing to maximise the production of *S. cerevisiae* biomass, as is the case in the industrial propagation of this organism for the provision of active yeast.

The industrial production of *S. cerevisiae* is nowadays performed through large-scale fed-batch fermentations, where a large amount of substrate is used in fed-batch manner in order to keep the concentration of the carbon substrate below the threshold that activates the Crabtree effect. In this way, respiration is privileged with its higher specific yield coefficient while maximising the amount of substrate that is used. Industrial fed-batch fermentation is well established and relies on a combination of predetermined growth curves and control-mechanisms based on sophisticated instrumentation.

The production of *S. cerevisiae* destined for a second fermentation in the bottle, as is the case for the preparation of Champagne, requires the propagation of yeast in alcoholic medium. In this way, the yeast is thought to adapt itself to ethanol, remaining active during the second fermentation and providing the fizz (carbonation) in the final product. This medium is largely toxic to the yeast and slows down the growth of the organism, hence the principle of fed-batch yeast propagation can be applied to both decrease ethanol toxicity and limit the Crabtree effect. The objective of this work was to design a simple and robust method for the production of yeast in alcoholic growth medium based on a simple and robust method to pilot the process.

Yeast growth is accompanied by heat production; in conventional bioreactors this heat is evacuated using different means that normally involve a conducting fluid. In this paper we will present a new method to control the feeding regime for a fed-batch yeast propagator. The process has the advantage that it can all be controlled using the simplest and most robust industrial probe available, that of temperature. The propagation strategy was developed at 5 l scale in insulated bioreactors and confirmed at pilot scale (800 l). At both scales, the propagation strategy showed a several-fold improvement over the batch method.

Keywords: Yeast propagation, Fed-batch propagation

Starmerella bacillaris and *Saccharomyces cerevisiae* Interactions During Alcoholic and Malolactic Fermentations

ORAL PRESENTATION ID: 160

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The diversity and complexity of wine environment during alcoholic and malolactic fermentation limits the successful prediction of wine characteristics. The use of selected starter cultures has allowed a better control of the fermentation process and the production of wines with established characteristics. Among them, the use of mixed fermentations with *Starmerella bacillaris* and *Saccharomyces cerevisiae* yeasts have gained attention in recent years due to the fructophylic nature of the first and the ability of this inoculation protocol to reduce the acetic and ethanol content of the wines. Both yeast species interact throughout the alcoholic fermentation and influence the chemical and aromatic composition of the wines. Many studies have been carried out to gain an insight to the nature of these interactions, with the aim to better control the wine fermentation.

Generally, successful inoculation protocol is considered the fermentation that enables *Starm. bacillaris* to dominate in the early stages of the fermentation process and demonstrate its peculiar characteristics, which are absent in *S. cerevisiae*. Inoculation delay between *Starm. bacillaris* and *S. cerevisiae* and combination of strains plays an important role to achieve this objective. Attention must be paid also to numerous winemaking variables, like nitrogen and oxygen availability and the presence of inhibitory or stimulator substances produced by the growth of yeasts. Among them, oxygen has positive impact on *Starm. bacillaris* by increasing its survival time and sugar consumption, while nitrogen sources could modulate the functional characteristics of the inoculated yeast strains to better control the fermentation process. Concerning the co-existence of both species during the fermentation process, we recently demonstrate that the early death of *Starm. bacillaris* in mixed fermentations with *S. cerevisiae* is not due to the depletion of nutrients not due to the production of toxic metabolites by the yeasts but rather to cell-to-cell contact mechanism, depend on the presence of viable cells of the last. Concerning malolactic fermentation, the consumption of nutrients by the above-mentioned yeast species and their produced metabolites may inhibit or stimulate the growth (and malolactic activity) of lactic acid bacteria (LAB), such as Lactobacillus plantarum and Oenococcus oeni. Specifically, yeast inoculation protocol and the combination of tested species/strains influenced LAB population dynamics, malic acid consumption and wine characteristics.

All these information's contributes to further understand *Starm. bacillaris* and *S. cerevisiae* interactions occurring during alcoholic and/or malolactic fermentations and allows a greater management of the production of specific metabolites to improve wine quality.

Keywords: Mixed cultures, interactions

The Indigenous Microbiota of Australian Aboriginal and Torres Strait Islander Fermentations

ORAL PRESENTATION ID: 234

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In Australia before the arrival of the first Europeans, Aboriginal people produced several fermented drinks including *Mangaitch* from flowering cones of a *Banksia* plant and *way-a-linah* from *Eucalyptus* tree sap. In the Torres Strait, Islanders learned from Filipinos how to make a fermented drink, Tuba, from coconut palm inflorescence sap. Despite having likely being used for many hundreds of years, the microbiology and chemistry of these processes is largely uncharacterised. Accordingly, microbial populations in soil, bark and sap samples from the *Eucalyptus gunnii* cider gum in Tasmania were evaluated by amplicon-based ITS phylotyping. Individual isolates from cider gum samples and from flowers, fruits and palm trees from Erub Island in the Torres Strait were identified. These isolates were then screened for tolerance to stress conditions found in high sugar environments and enzymatic activities potentially relevant to these. A diverse assortment of yeast species was found, with clear differences in population make-up between the two main sources and their inherent conditions. The nature and properties of these yeast isolated is discussed.

Keywords: Mangaitch drink, Fermented drink

New Ingredients for the Fermentation of Beer: Non-*Saccharomyces* Yeasts from Wine

ORAL PRESENTATION ID: 135

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The craft beer industry has been increasing during recent years and it is an important sector in Spain. The industry has been focused on *Saccharomyces* yeast, but there is an increase of the studies and the use of non-*Saccharomyces* yeast in many fermentative food industries (wines, beer, bread, etc.). *Brettanomyces*, *Hanseniaspora*, *Lachancea*, *Torulaspora* and *Wickerhamomyces* genera have been studied as alternative species for the production of beer. They open new possibilities to modulate flavour and other sensory properties during fermentation. The aim of this study is the application of non-*Saccharomyces* yeast for the production of pale ale beer.

To gain this objective we used the bank of native wine yeast from D.O. “Vinos de Madrid”. We tried it at laboratory scale to select the best yeasts and then to test it at industrial scale. Furthermore, these strains will be used for mixed culture fermentations. At laboratory scale we have analysed the fermentative kinetic, production of foam, ability of yeasts to ferment maltose, production level of hydrogen sulphide (H2S), aromas, melatonin production and alcohol, lactic acid, colour, bitterness and residual fermentable sugars (CDR BeerLab equipment).

We have tested 102 non-*Saccharomyces* yeast strains from 33 different species, which only 62 were able to finish the fermentation. A preselection of 42 strains was made based on their brewing process good characteristics. The aromatic profile was analysed with a group beer tasting experts and gas chromatography was used to identify relevant aromas in these beers. The production of melatonin was analysed with liquid chromatography. Finally, 10 yeasts were selected for analysis at the brewery and to confirm laboratory scale results. At the same time, these first 42 strains selected are going to be used for mixed fermentation.

In general, about 50% of the strains studied are able to ferment beer wort. They show fermentative kinetics, foaming capacity, melatonin production and a similar use of sugars to the commercial yeast strain used in the brewery. However, not all the beers obtained have been adjusted to the flavours expected in pale ale style.

Keywords: Native strains, Non-*Saccharomyces*

Physiological Role and Industrial Opportunities of Yeast Aroma Production

ORAL PRESENTATION ID: 106

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Yeast cells are often employed in industrial fermentation processes for their ability to efficiently convert relatively high concentrations of sugars into ethanol and carbon dioxide. Additionally, fermenting yeast cells also produce a wide range of other compounds, including various higher alcohols, carbonyl compounds, phenolic compounds, fatty acid derivatives, and sulfur compounds. Interestingly, many of these secondary metabolites are volatile and have pungent aromas that are often vital for the end product quality. However, the physiological role of aroma formation by yeast cells has been elusive. We have generated many new yeast hybrids with different aroma profiles that can be used to expand the spectrum of aroma's in fermented beverages. Moreover, our research shows that yeasts aroma's serve to attract fruit flies, that in turn serve as vectors for the yeast cells.

Keywords: Aroma, Isoamyl acetate, Drosophila, Ecology

SESSION 4: Probiotic Yeast

Industrial Production of Baker's Yeast – Current Trends and Challenges

SPONSOR SPEECH ID: 237

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Baker's yeast, which constantly evolved since its first discovery, has gained many varieties today. While the use of yeast in bakery was initially limited only to the leavening of dough as much as possible, with the advent of industrial fermentation, different yeast strains for each type of dough, such as sugared, frozen and aromatic have been developed. For the improvement of yeast strains, breeding as a traditional method has now been replaced by more scientific approaches such as genetic shuffling, directed evolution and mutagenesis. Today, strains developed via very precise DNA modifications are moving rapidly to the point where they can be considered completely safe. At the core of all strain improvement methods, selection of the right strain among several candidates is critical. Advances in robotic and analytical technologies enabled on-line monitoring and screening of various parameters in hundreds of micro-scale fermentations. In addition to on-line monitoring, with the further development of high-precision pumping and injection systems, it will be possible to transfer different operational modes, which can be achieved by active process control at the micro-scale, to large-scale systems in very short time. Thus, this presentation will discuss an overview of available technologies for strain development from classical to newly adopted high-throughput systems and will present cases where we employed yeast technologies in various fields.

Keywords: Baker's Yeast, Strain Development, Upstream, Fermentation Process

Beneficial Impact of Food-Borne and Probiotic Yeasts on Human Health – Traits That Determine Their Biological Functionality

ORAL PRESENTATION ID: 107

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Yeasts do not only play a significant role in the technological production of many fermented food and beverages, they also offer nutritional improvements of the products. Especially for cereal-based foods, yeasts play a significant role in the degradation of anti-nutritional factors as e.g. phytic acid which due to chelation of cations such as Fe^{2+}, Zn^{2+}, Ca^{2+} and Mg^{2+} limits their bioavailability. Yeasts species as e.g. *Pichia kudriavzevii* have been reported to have high phytase activity, though significant variations occur at the intra-species level. Various yeast species as e.g. *Saccharomyces cerevisiae* are additionally able to produce folate (vitamin B9) and likewise huge variations are observed at both species and strain levels.

Food-associated yeasts and yeasts administered as dietary supplements or probiotics do additionally have the potential to impact human health through various forms of interactions. Specifically, *Saccharomyces boulardii* (taxonomically acknowledged as belonging to *S. cerevisiae*) has been shown to have a positive impact on the treatment of acute infectious diarrhoea by restoring the normal endogenous digestive microbiota and is specifically used in the treatment and prevention of recurring *Clostridium difficile* associated diarrhoea, an effect mediated through proteolysis of the *C. difficile* toxins. Also, probiotic yeasts have been used in the treatment of inflammatory bowel diseases such as Crohn's disease and ulcerative colitis, indicating an ability of *S. boulardii* to influence human immune responses. Specifically, probiotic yeasts have been proven to lower the pro-inflammatory response upon infection with various pathogens.

Though it is currently accepted that our gastrointestinal tract contains an overwhelming number of microorganisms which have an increasingly recognized impact on human health, our knowledge on how yeasts can influence human health in a positive manner is currently rather scattered. The aim of the presentation is therefore to give an overview on existing knowledge within the area and to present a number of scientific results dealing specifically with the effect of yeasts on improvement of nutritional quality of fermented foods, their interactions with the human immune system and their potential role as future probiotics.

Keywords: Probiotic yeasts, Anti-nutritional factors, Immunological responses, *Saccharomyces boulardii*

Unique Genetic Basis of the Distinct Antibiotic Potency of High Acetic Acid Production in the Probiotic Yeast *Saccharomyces cerevisiae* var. *boulardii*

ORAL PRESENTATION ID: 122

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The yeast *Saccharomyces boulardii* has been used world-wide as commercial probiotic, but the basis of its probiotic action remains obscure. It is the only yeast prescribed against gastrointestinal diseases. Studies in animal models and clinical trials in patients have shown its effectiveness against many gut-related diseases, including Crohn's disease, ulcerative colitis, Antibiotic Associated Diarrhoea, gut inflammatory manifestations in HIV patients, recurrent *Clostridium difficile* infections and diarrhoea as a result of infections by bacterial enteropathogens. The origin of *S. boulardii* can be traced back to south-east Asia, where it was first isolated from lychee fruits in 1920 by Henry Boulard. Probably all *S. boulardii* strains available nowadays are derived from an original strain isolated by Mr. Boulard. *S. boulardii* is considered conspecific with budding yeast *S. cerevisiae*, which is generally used in classical food applications. They have an almost identical genome sequence, making the genetic basis of probiotic potency in *S. boulardii* puzzling.

We now show that *S. boulardii* produces at 37°C unusually high levels of acetic acid, which are strongly inhibitory to bacterial growth in agar-well diffusion assays and could be vital for its unique application as probiotic among yeasts. Using pooled-segregant whole-genome sequence analysis with *S. boulardii* and *S. cerevisiae* parent strains, we succeeded in mapping the underlying QTLs, and identified mutant alleles of *SDH1* and *WHI2* as the causative alleles. Both genes contain a SNP unique to *S. boulardii* (*sdh1*^{F317Y}and *whi2*^{S287*}) and fully responsible for its high acetic acid production. *S. boulardii* strains show different levels of acetic acid production, depending on the copy number of the *whi2*^{S287*}allele.

Our results offer the first molecular explanation as to why *S. boulardii* could exert probiotic action as opposed to *S. cerevisiae*. They reveal for the first time the molecular-genetic basis of a probiotic action-related trait in *S. boulardii* and show that antibacterial potency of a probiotic microorganism can be due to strain-specific mutations within the same species. We suggest that acquirement of antibacterial activity through medium acidification offered a selective advantage to *S. boulardii* in its ecological niche and for its application as probiotic. Hence, our work provides a possible explanation for the selection of *S. boulardii* as only probiotic yeast as opposed to the very closely related, likely conspecific *S. cerevisiae* strains.

Keywords: *Saccharomyces boulardii*, Probiotic, Acetic acid production, Antibacterial potency, Polygenic analysis

Kombucha Fermentation: Yeast Populations and Biological Activities

ORAL PRESENTATION ID: 151

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Kombucha is a traditional fermented tea supposed to bring health benefit to consumers. The fermentation is carried out by a complex symbiotic consortium of bacteria and yeasts called SCOBY. The composition of Kombucha as well as the management of the fermentation is not yet well characterized and can vary according to the SCOBY used to start the fermentation. In this work we focus on the yeast populations and compared three different starters in the same conditions of fermentations.

Black tea infusions were fermented for 3 weeks by the 3 consortiums. During this time total yeasts populations was monitored by plate counting. The dominant yeasts were identified by shotgun sequencing. Main biological activities, antioxidant and anti-inflammatory, were determined in-vitro on final butanol and ethyl acetate extracts.

The fermentations rates were very different according to the starters used varying from 2 to more than 3 weeks. The total yeasts population varying from1 to 50 million of cells by ml. In all cases the predominant identified yeasts were *Brettanomyces*. An interesting IC50 value of 7 µg/mL was obtained against the DPPH radical for the antioxidant activities of all the samples. On the opposite the anti-inflammatory activities were different according to the Scoby.

As a conclusion the kombucha fermentations behaviour seems to be very different according to the starters used despite the fact that the yeast populations distribution is very similar.

Keywords: *Brettanomyces*, Scoby, Antioxidant

Production of Different Yeasts on Grown on Media Composed of Spruce Sugars and Protein Hydrolysates from Poultry By-Products

ORAL PRESENTATION ID: 171

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Due to the possible future shortage of feed protein, alternative protein sources that can replace conventional soymeal or fishmeal need to be explored. Several large industrial organic side-streams could potentially be upgraded to feed protein using fermentation process to generate microbial protein. Yeast is the most widely accepted microorganism for production of single cell protein, because of its superior nutritional quality and acceptability among consumers. In this study, we have assessed the growth of four different yeasts, *Cyberlindnera jadinii*, *Wickerhamomyces anomalus*, *Blastobotrys adeninivorans* and Thermosacc® Dry (*Saccharomyces cerevisiae*), on media composed of enzymatically saccharified sulfite-pulped spruce wood and hydrolysates of by-products from chicken and we characterized the yeast biomass.

Generally, the yeast grew very well on the spruce- and chicken-based medium. *B. adeninivorans* stood out as the most versatile yeast in terms of nutrient consumption and yields with 0.9 g cells and 0.5 g protein per g of sugar, indicating utilization of other carbon sources in the medium. The next best performing yeast in terms of yield was *W. anomalus* with up to 0.6 g cells and 0.3 g protein per g sugar. Comparative compositional analyses of the yeasts revealed favourable amino acid profiles that were similar to the profiles of soymeal, and even more so, fish meal, especially for essential amino acids.

The efficient conversion of industrial biomass streams to yeast biomass demonstrated in this study opens new avenues towards better valorization of these streams and development of sustainable feed ingredients. Furthermore, we conclude that production of *W. anomalus* or *B. adeninivorans* on this promising renewable medium may be potentially more efficient than production of the well-known feed ingredient *C. jadinii*. Further research should focus on medium optimization, development of semi-continuous and continues fermentation protocols and exploration of downstream processing methods that are beneficial for the nutritional values of the yeast for animal feed.

Keywords: Microbial protein, Spruce, Enzymatic hydrolysis, Protein hydrolysate, Aquaculture

From Strain to Product

ORAL PRESENTATION ID: 117

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Yeast is unique in its many applications and uses. It can be used as a nutritional additive, it can leaven dough and ferment foodstuffs, it can make biofuels, it can be used to make heterologous proteins and much, much more. University groups all over the world are finding new ways to harness the power of yeast every year, either by isolation from interesting places, directed evolution, mutagenesis, or genetic modification.

But, when a new strain has been found that has great potential in a new application, how can it be made into a commercial product? Here we show that the production process itself poses different challenges to those in the applications. Large-scale production, processing, packaging, storage and transport create large fluctuations in nutrient availability, inhibitor concentrations, osmotic pressure, temperature and water activity, as evidenced by substrate and process analyses. The yeast needs to be able to withstand all these fluctuations during the process as well as be able to cope with the often completely different conditions once it reaches the application.

AB Biotek, a division of AB mauri, specializes in bringing to market new yeast strains by using scaled down versions of the commercial processes with a high level of flexibility to try out different adjustments as well as close collaborations with strain development laboratories. Data are presented that show the improvements that can be made with proper protocol development. This way every opportunity for yeast to make the world a better place gets the best possible chance of coming to life.

Keywords: Strains, Commercialisation, Production, Stress

SESSION 5: Yeast Taxonomy, Ecology and Biodiversity

Polar and non-Polar Cold Habitats: Little Explored Habitats for Fungal Life

ORAL PRESENTATION ID: 192

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Although their extremely harsh environmental conditions, polar and non-polar cold habitats are compatible with the life of psychrophilic and psychrotolerant microorganisms, which have developed specific mechanisms of adaptations to overcome the adverse effects of low temperatures on cell physiology. However, recent studies have reported that the impact of global warming on polar and non-polar cold habitats (e.g. Antarctica, Alpine glaciers, etc.) is amplifying every year. Therefore, those habitats can be considered sentinel areas for monitoring cold-adapted microbial biodiversity. Hence, the study of the diversity of fungal (yeasts and filamentous fungi) populations occurring in polar and non-polar cold areas may be considered strategic for increasing the knowledge on the microbial ecology of these understudied niches.

Samples of permafrost, ice, brines and debris were collected from Antarctic and Alpine sites. Fungal (yeasts and filamentous fungi) diversity was investigated via NGS targeting the ITS region (Illumina Miseq). Bioinformatic analysis of sequence data was performed using Qiime 2 (https://qiime2.org/). Analysis of alpha- and beta-diversity, Linear discriminant analysis Effect Size (LEfSe) and Pearson correlation were performed for studying the taxonomic assemblage of fungal communities and the interaction between chemical/physical parameters and fungal operational taxonomic units (OTUs, classified at the genus level).

OTUs attributable to *Ascomycota* predominated among filamentous fungi, while *Basidiomycota* dominated among yeasts. At the phylotype level, yeasts dominated the fungal communities: the most frequently found genera were *Candida*, *Leucosporidium*, *Malassezia*, *Naganishia* and *Sporobolomyces*. Overall, the analysis of alpha- and beta-diversity revealed a high phylogenetic differentiation among samples even at the small-scale level. Salinity, conductivity, pH and the concentration of organic carbon showed a significant ($p < 0.05$) impact on the abundance of some filamentous and yeast genera.

The high alpha- and beta-diversity of yeasts and filamentous fungi confirm that polar and non-polar cold habitats can be considered a notable hotspot of fungal cold-adapted diversity.

Keywords: Cold-adapted fungal biodiversity, Psychrophilic/psychrotolerant yeasts

The Frequency of Transgressive Segregants in Interspecific *Saccharomyces* Hybrids

ORAL PRESENTATION ID: 123

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Intraspecific hybrids are common in the budding yeast genus *Saccharomyces*, producing recombined genomes with a large range of ploidies. Interspecific hybrids harbor large amounts of adaptive variation from which transgressive segregants may emerge, showing larger fitness than their parents in new environments. Despite a growing interest of agronomy in these hybrids, especially for wine making, we know very little about the underlying phenotypic and genetic mechanisms causing transgression.

In this study, we made divergent F1 and F2 hybrids crosses including all *Saccharomyces* sensu stricto species. First, we made phenotypic growth profiles for each parent in 7 different stressful environments, mimicking natural, fermentation process, pollutant or oxidative environments. Then we compared the fitness of hybrids to their parents and counted the number of transgressive F2 segregants in all environments. We found that F1 hybrid fitness was generally predicted by the growth phenotypes of the better parent. Transgression frequency in F2 segregants was correlated with the genetic distance of the parental species. However, F2 transgression frequency was not correlated to the phenotypic distance between species, nor to F1 fitness. Finally, we found that transgression frequency strongly varied between test environments. Improving genomes through hybridization is a process widely used for the design of industrial strains. Our data can be informative for the optimization and experimental evolution of strains exposed to specific environmental stressors.

Keywords: Transgressive, Hybrid, Evolution

Buffalo rumen: A Potential Niche for Novel, Thermotolerant and High Ethanol Producing Yeasts

ORAL PRESENTATION ID: 177

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Bioethanol is the most widely used biofuel and has a long history as an alternative fuel. Yeasts play an essential role in bioethanol production by fermenting a wide range of sugars to ethanol. Typical yeast ethanol fermentations require 30°C to 35°C. In tropical countries like India, maintaining the low operating temperatures requires expensive cooling systems. Significant cost savings can be achieved if the fermenter is kept at or above 45°C. This would require thermotolerant yeasts which can produce ethanol at high temperature using simultaneous saccharification and fermentation. However, very few thermotolerant yeasts have been reported so far. Therefore, we aimed to explore habitats from which more thermotolerant yeasts can be isolated. The gut of ruminants is one such niche, wherein the temperature of rumen is 39.6°C and might harbour yeasts which would be thermotolerant and also aid in digestion of lignocellulosic biomass. Hence, the objective was to isolate and characterize thermotolerant yeasts from buffalo rumen and assess their ethanol producing potential at high temperatures.

Rumen fluid was collected from buffalo gut at a slaughterhouse in Pune, Maharashtra. Thermotolerant yeasts were isolated at 42°C using suitable media. The purified yeast cultures were characterized by a combination of microscopic morphological characteristics, biochemical analysis and molecular phylogenetic analysis. Representative strains were assessed for ethanol producing potential at 30°C, 40°C and 45°C.

A total of 46 yeasts were isolated and identified which belonged to ten species. Yeasts like *Candida nivariensis, Cyberlindnera fabianii, Tortispora caseinolytica, Kluyveromyces marxianus, Metschnikowia koreensis* and *Meyerozyma caribbica* are being reported for the first time from buffalo rumen. Based on growth temperature, sugar assimilation profile, and ethanol production capabilities, 12 out of 46 yeast strains were shortlisted for assessment of ethanol production at higher temperature and optimization studies. It was observed that ten yeasts yielded high levels of ethanol at 40°C (3.42 - 4.09 g/L) with 1% glucose.

The present study highlights buffalo rumen as a niche for novel yeast species and thermotolerant yeasts, which are capable of producing ethanol at high temperatures. This investigation emphasizes that buffalo rumen is associated with high yeast diversity and needs further exploitation for isolation of more thermotolerant yeasts. The ethanol producing yeasts are being tested for high temperature ethanol production from lignocellulosic biomass, which would be a sustainable means of ethanol fermentation at industrial level.

Keywords: Rumen, Thermotolerant yeasts

Membrane Engineering to Improve *Saccharomyces cerevisiae* Robustness Towards Formic Acid

ORAL PRESENTATION ID: 198

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Lignocellulose is one of the most abundant renewable feedstocks on the planet and thus a desirable biomass for bio based microbial processes. However, the utilization of lignocellulosic biomasses require a pretreatment phase for the release of fermentable carbon sources. Simultaneously, a broad range of microbial inhibitors, among which weak organic acids (WOAs), are also released. WOAs enter in the cell by simple diffusion, especially at low pH, and once inside the cell, at the cytosolic pH, they dissociate into proton and the corresponding anion. The accumulation of both protons and anions inside the cell compromises metabolism and can even cause growth arrest. Formic acid is one of the main WOAs released from lignocellulosic biomasses at concentrations around 1.5 g/L. Despite it is not the most abundant WOA, its small molecular size results in a higher toxicity to the cells compared to other longer WOAs. At the same time, WOAs, such as lactic acid, can also be products of interest obtained from microbial factories to be used in several industrial sectors: food and beverage, cosmetics, pharmaceutics, chemicals. Therefore, controlling the cellular inward/outward flux of WOAs is crucial for the development of more efficient cell factories. Therefore, plasma membrane engineering has been envisaged as a strategy to increase the cell performance towards WOAs.

Since *Saccharomyces cerevisiae* is widely used as a cell factory for the production of numerous compounds starting from renewable feedstocks, here we evaluate the capacity of a wild type strain and of a strain engineered for the production of lactic acid to cope with the stress caused by formic acid alone or in combination with the lactic acid produced. Furthermore, to increase the strain(s) robustness, we are evoking a membrane rewiring using a global transcription machinery engineering approach, focusing on the modulation of the transcription factor (TF) *ECM*22, involved in the regulation of ergosterol biosynthesis.

Our data suggest that the deletion of *ECM*22 has a positive impact on yeast robustness in the presence of formic acid.

We will illustrate how the increased robustness correlates with a rearrangement of the plasma membrane composition and function, in terms of lipids and proteins and how this can impact lactic acid production.

Keywords: *Saccharomyces cerevisiae*, Membrane engineering

The Effect of Backslopping on the Yeast Diversity of Tarhana Fermentation

ORAL PRESENTATION ID: 204

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Tarhana is a traditional fermented food which is manufactured by wheat flour, yogurt, sourdough and various vegetables and spices (tomato, red pepper, onion, peppermint, salt, etc.) that the resulting dough is fermented and subsequently dried and grinded. This cereal-based food is consumed as an instant soup with high nutritional value. One of the fermenting microflora at tarhana fermentation is yeasts which are responsible for leavening as well as enhancing the aromatic structure. Tarhana fermentation is driven by the lactic acid bacteria and yeasts with less extent. Yeasts are contaminated from the ingredients especially wheat flour. However, tarhana is produced differently every production cycle due to the imbalance of the yeast diversity. Backslopping is a traditional inoculation procedure based on the addition of a small volume of a previously fermented product to control the fermentation process. The aim of this study was to evaluate the effects of backslopping applied at different temperatures (25 and 30 °C, pH (3.70 and 4.00) and inoculation rates (5, 10 and 15%) on the diversity of yeasts during fermentation of tarhana.

The yeast amount of all tarhana dough changed between 3.15-4.12 log CFU/g at initially and increased above to 6.00 log CFU/g at 25 °C, however they remained below at 30 °C which were backslopped at both 3.70 and 4.00 pH. The yeast amount was lower at backslopped tarhana dough samples incubated at 30 °C than 25 °C. But, the backslopping pH did not significantly affect the yeast amount in dough samples. PCR-DGGE analysis showed that backslopping at 4.00 pH resulted richer yeast diversity than 3.70 pH at both fermentation temperatures. Additionally, the fermentation yeast profile of tarhana dough samples were different at 25 °C and 30 °C. When the steady conditions were achieved after backslopping, *Saccharomyces*, *Candida*, *Pichia* and *Kazachstania* dominated the dough fermentation independently from the backslopping parameters. Especially *Candida humilis* was the common and distinct species at all tarhana dough samples.

In conclusion, backslopping stabilized the yeast prevalence and diversity at tarhana fermentation and maintained steady yeast microflora at every fermentation cycle. Accordingly, 25 °C and 3.70 pH could be proposed for the production of tarhana with backslopping.

Keywords: Tarhana Fermentation, Yeast diversity, Backslopping

Acknowledgements: This work was supported by the Scientific Research Projects Unit of the University of Cukurova with the Project number FDK-2017-7769.

SESSION 6: Yeast Taxonomy, Ecology and Biodiversity

Adaptive Laboratory Evolution to Enhance Organic Acid Tolerance in *Kluyveromyces marxianus* on Residual Biomass

ORAL PRESENTATION ID: 197

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The concept of biorefineries is revolutionising the production industry in order to sustainably meet the basic requirement of growing population with limited resources. In biorefineries one of the key instruments in the biochemical conversion of lignocellulosic feedstocks (the most abundant renewable biomass present on the planet) to advanced biofuels and other commodities is the development and usage of efficient, robust, versatile microbial cell factories with innate or engineered traits. Regrettably, the pretreatment of lignocellulosic biomass, necessary to make the sugars accessible, releases organic acids that are inhibitory to the production microorganisms and *Kluyveromyces marxianus*, a non-*Saccharomyces* yeast, is not an exception despite several advantages that it possesses. It has unique ability to grow at high temperatures (up to 45ºC) and to utilize broad range of substrates, including the C5 sugars that are present in lignocellulosic biomass.

In this work, we aim to enhance the organic acid tolerance of *K. marxianus* on sugar beet pulp (SBP), a residual lignocellulosic biomass composed of C5 and C6 sugars along with acetic and lactic acid that inhibit growth at low pH. Low pH is an important parameter in industrial production to minimize bacterial contamination and to ease the purification of organic acids when these are the desired end products.

Adaptive Laboratory Evolution (ALE) was used to generate and select *K. marxianus* variants with improved tolerance to weak organic acids at low pH in SBP at two different temperatures. ALE was performed by sequential serial passages in shake flasks every 24 hours.

ALE was performed for up to 1805 and 1522 generations at 30ºC and 40ºC respectively over a period of 105 days. The performances of wild type strain and evolved isolates were tested by pH-gradient acetic acid plate assays and growth kinetics in liquid media. By comparing the specific growth rate and the duration of the lag phase, *K. marxianus* variants with improved tolerance were selected.

This work describes a strategy to obtain *K. marxianus* yeast strains with improved tolerance to organic acids present in lignocellulosic feedstocks for use in biorefineries. Genome sequencing will help in identifying the mutations involved, thereby better describing how their desirable phenotypic traits can be linked to their genotype. The best- evolved cell factory will be engineered for production of organic acid such as lactic acid, which has wide range of applications in various industrial sectors.

Keywords: *Kluyveromyces marxianus*, Adaptive laboratory Evolution

Fungal Diversity Based on Ortholog Analysis of Draft Genomes

ORAL PRESENTATION ID: 193

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Genomic data are useful for constructing a reliable molecular phylogenetic tree (backbone tree) and identifying phenotypic/genomic information that distinguishes one clade from the others. We showed that presence-absence matrix analyses of all orthologous genes is useful for delineation between the genus levels using the genomes of the species in *Trichosporonales* (*Agaricomycotina, Basidiomycota*), previously. We also found the 77 orthologous groups (OGs) common to all strains in the *Trichosporonaceae*, assuming that they represent *Trichosporonaceae*-specific genes. In this study, we prepared the larger dataset and performed the presence-absence matrix analysis focusing on the distinctiveness of *Saccharomycotina* in *Ascomycota*.

Ascomycota is phylogenetically divided into three groups; *Saccharomycotina*, *Taphrinomycotina* and *Pezizomycotina*. Species of *Saccharomycotina* and *Taphrinomycotina* proliferate by budding or fission of yeast cells, while most species of *Pezizomycotina* are filamentous and some species called black yeasts belong to *Pezizomycotina*. We created a dataset consisting of ca. 200 fungal genomes for the analysis. As the number of genome available species is small in *Taphrinomycotina*, we mainly analysed the relationship between *Saccharomycotina* and *Pezizomycotina*.

The presence-absence matrix analysis of OGs showed that *Saccharomycotina* is distinct from *Pezizomycotina*, which is congruent with the phylogeny of *Ascomycota*. The usefulness of presence-absence matrix analyses is confirmed not only in the genus level but also in the higher taxa of fungal taxonomy. The accumulation of such data will contribute to comprehensive understanding for fungal diversity.

Keywords: Orthologous gene, Yeast taxonomy

DNA Barcoding of Yeast Strains from Anatolia

ORAL PRESENTATION ID: 184

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Since the agriculture of grapes and application of wine making in Anatolia dates back to 4000 B.C., it is important to isolate and identify of *S. cerevisiae* strains belong to Anatolian vineyards. For this purpose, new rapid, high-throughput and reliable identification methods are required. In here, we described a combined approach based on two high-throughput techniques in order to improve the identification of yeast strains. MALDI-TOF MS analysis and molecular DNA barcoding with ITS, LSU and RPB2 marker regions were applied to identify 120 strains belonging to *S. cerevisiae* and other yeast strains from different sources and areas (commercial cultures, collection cultures, universities research cultures, grape and soil samples from Central Anatolia Region cities). The classical (macroscobic, microscobic and biochemical) and molecular definitions of featured yeast isolations are made. First one hundred and twenty yeast isolates were analyzed by MALDI-TOF MS; second they were subjected to DNA barcoding. MALDI-TOF results of these 120 strains confirmed 64 of them as *S. cerevisiae*. When the molecular evaluations of these strains were completed using BLAST, it was found out that MALDI-TOF MS results and DNA barcoding results showed %98.87 similarity for *S. cerevisiae* strains. The result of study showed that ITS, LSU and RPB2 regions can be used with a consistent performance in inter-species and intra-species level identification of *S. cerevisiae*. Results suggested that the identification success increase with the combined approach based on MALDI-TOF MS and DNA barcoding.

Keywords: DNA barcoding, *S. cerevisiae*, Yeast

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Intracellular Protective Reactions in Yeasts at Stress Conditions

ORAL PRESENTATION ID: 104

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Studies of mechanisms of yeast cells survival in the extreme conditions of the environment are important for both - biotechnology and medicine. There is the unique possibility to reveal different intracellular protective reactions in these studies and obtained information will facilitate to the development of efficient approaches for 'artificial' increase of eukaryotic cell stability at various biotechnologies. Our studies revealed that main intracellular protective reaction in yeasts under osmotic and dehydration stresses are condensation of chromatin in nucleus, DNA in mitochondria and folding of plasma membrane by the development of initially existed small folds. Comparison of *Saccharomyces cerevisiae* cells with osmotolerant yeast *Debaryomyces hansenii* which appeared to be more resistant to dehydration then baker's yeast showed the differences in membrane lipids phase transition temperatures which are essentially lower in osmotolerant yeast. Special pre-treatments of yeast led to the synthesis in the cells of various sugar alcohols which decrease the lipids phase transition temperatures like it is normally provided by trehalose. It is supposed that in the case of absence of trehalose sugar alcohols may substitute it for the protection of the molecular state of intracellular membranes. If the trehalose was accumulated but in the non-sufficient amount sugar alcohols provide additional protection for the most important macromolecules. Recent studies confirmed the importance of glutathione synthesis in the cells and especially in the case of genetically engineered yeasts which are not resistant to many extreme factors including dehydration. Comparison of mesophilic and thermotolerant strains of *S. cerevisiae* showed that thermoresistance is accompanied also with higher yeast cells stability at dehydration conditions. Essentially higher amount of Hsp70 protein was revealed in the thermoresistant yeast strain cells. In the studies of psychrophilic yeasts *Solicoccozyma terricola* and *Naganishia albida* it was found that they are extremely resistant to dehydration. Further research showed that the amount of Hsp70 protein in the cells of these yeasts was at least 3 times higher than in the resistant strains of baker's yeast. It was concluded that this protein also is very important for yeast cells resistance to various extreme factors of the environment. Conclusions made in these studies gave the possibility to improve efficiency of some biotechnological processes including also waste-less production of bioethanol and some other valuable compounds.

Keywords: Anhydrobiosis, Dehydration-Rehydration, Protective compounds

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Ethanol and Biomass Production by Newly Isolated Wild-Type Yeast Strains Cultivated on Glucose in Shake-Flask Experiments

ORAL PRESENTATION ID: 243

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Aim of the present investigation was to assess the potential of several newly isolated yeast strains to perform growth and potential ethanol production under aerobic conditions during cultivations on glucose, being positive thus in the "Crabtree effect", in trials performed in shake-flask nitrogen-excess media. Several yeasts (*Candida boidinii, Candida oleophila, Candida tropicalis, Metschnikowia pulcherrima, Williopsis saturnus* and *Pichia ciferrii*) were chosen whereas the positive reference on Crabtree effect was a *Saccharomyces cerevisiae* strain and the negative one was the typical oxidative non-conventional yeast *Yarrowia lipolytica*. With the exception of *Pichia ciferrii* (and, certainly, *Yarrowia lipolytica*) all strains rapidly assimilated glucose and converted it into ethanol, despite aerobic conditions imposed, being, Crabtree-positive. Low DCW quantities were produced at the first growth step, while the majority of the Crabtree-positive yeasts produced non-negligible total polysaccharide quantities per unit of DCW at the very early growth phases, which coincided with significant glucose uptake and ethanol secretion. Dissolved oxygen concentration values (DOC, % v/v), equally rapidly were depleted at the first fermentation steps reaching to values 10-20% v/v. Maximum ethanol quantities recorded varied between 17-24 g/L. Rapid glucose exhaustion resulted in graduate ethanol re-consumption (ethanol "make-accumulate-consume" effect) that coincided with significant decrease in total cellular polysaccharides values (in % per DCW). Simultaneously, at this late growth step, in most cases cellular lipids in DCW values somehow increased reaching values of lipids in DCW up to 19.1% w/w. In contrast to the Crabtree-positive strains, Crabtree-negative microorganisms (*Yarrowia lipolytica* and *Pichia ciferrii*) produced clearly higher DCW values and assimilated less rapidly glucose from the medium. Cellular lipids rich in the fatty acids oleic and linoleic, were recorded for all available yeasts strains.

Keywords: Ethanol and biomass production

Acknowledgement: The current investigation was financially supported by the project entitled "Exploitation of new natural microbial flora from Greek origin amenable for the production of high-quality wines" (Acronym: Oenovation, project code T1EΔK-04747) financed by the Ministry of National Education and Religious Affairs, Greece (project action: "Investigate – Create – Innovate 2014-2020, Intervention II").

SESSION 7: Yeast Genetic and Genomic

NGS for Industrial Yeast Development at AB Biotek

SPONSOR SPEECH ID: 116

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AB Biotek (ABB), a division of the global yeast manufacturer AB Mauri (ABM), works with yeast and other microbes from the ABB/ABM culture collection to develop new products for markets ranging from beverage alcohol, to animal and human nutrition, to biofuels. As part of its new product development, ABB uses next generation sequencing methodologies and has experimented with use of RNAseq as a tool to identify expressed multilocus sequence (eMLS) markers to inform our work on yeast and other microbes for new product development.

RNAseq and whole genome sequencing (WGS) was performed with partners. Briefly, for RNAseq, RNA was extracted from cells at different time points and sequenced on an Illumina platform with approximately 30,000,000 reads per sample. Sequence alignments were made to a reference genome and expression levels of the transcripts calculated (RPKM). For WGS, DNA from yeast was sequenced on a PacBio platform with >400× coverage, and de novo assemblies produced using CANU and FLYE. It was not possible to output fully phased, diploid genomes.

Although ABB uses WGS to characterize strains in its culture collection, we have found increasing utility from RNAseq data from yeast responding to fermentation conditions mimicking the intended application for the yeast product. ABB has developed expressed multilocus sequence (eMLS) markers from those hundreds to thousands of differentially-expressed genes during fermentation to the intended product (e.g. bioethanol, bread). The eMLS markers are used to determine gene expression profiles and strain phylogeny, track alleles in strain development projects, and provide insight into the diversity of gene alleles represented in the entire culture collection. The sum of all the expressed RNAs (eMLS markers) provides the ability to rapidly type new strains based on data from multiple loci of interest.

The eMLS markers allow for rapid, low-cost phylogenetic characterization of strains, empowers strain development, and highlights allelic differences which impart differentiated characteristics to ABB and ABM strains.

Keywords: Sequencing, NGS, Genome, RNAseq, WGS

Transcriptional Control Limits Central Carbon Metabolism of Crabtree Negative Yeasts: A Potential Role in Early Evolution of Fermentation?

ORAL PRESENTATION ID: 247

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The Crabtree phenotype defines whether a yeast can perform simultaneous respiration and fermentation under aerobic conditions at high growth rates, a phenomenon that resembles the Warburg effect in cancer cells. It is assumed that this phenotype evolved in yeasts with the first fruit plants about 125-150 million years ago, providing Crabtree positive yeasts an evolutionary advantage in novel sugar rich environments. It is still under debate whether the advantage lies rather in the competition to other microorganisms or in a solution for metabolic imbalances due to high sugar supply. Whole genome duplication, global promoter rewiring and loss of respiratory complex I are the main molecular events that contributed to the evolution of the Crabtree effect in budding yeasts. All these events enhanced the strength of the Crabtree effect, however they cannot explain how an initial evolutionary driving force could have emerged to establish a selective force.

Here we show that overexpression of a single Gal4-like transcription factor is sufficient to convert Crabtree-negative *Komagataella phaffii* (*Pichia pastoris*) into a Crabtree positive yeast. Upregulation of the glycolytic genes and a significant increase in glucose uptake rate due to the overexpression of the Gal4-like transcription factor caused an overflow metabolism, triggering both short-term and long-term Crabtree phenotypes. This indicates that a single genetic perturbation leading to overexpression of one gene may have been sufficient as a first molecular event towards respiro-fermentative metabolism in the course of yeast evolution.

Implications on evolutionary scenarios of fermenting yeasts will be discussed, as well as potential applications for low alcohol fermented beverages.

Keywords: Respiratory fermentation, Crabtree effect, Evolution

Evolutionary Engineering and Molecular Characterization of Stress-resistant Yeasts Using Systems Biology Tools

ORAL PRESENTATION ID: 167

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During industrial bioprocesses, yeast cells are exposed to a variety of stress types. Thus, to have robust strains that are multi-stress resistant is highly desirable. To this end, our research is focused on improving robustness or resistance of yeast cells to different stress types such as ethanol, freeze-thaw, oxidative/metal stresses, as well as to the inhibitors of lignocellulosic ethanol fermentations by using evolutionary engineering, an inverse metabolic engineering strategy. The ultimate goal is to gain insight into the complex molecular basis of the stress-resistant yeast phenotypes using a systems biology approach that involves transcriptomic, genomic and proteomic analyses.

For each stress type, batch selections were applied to a chemically mutagenized, genetically diverse initial population under selective stress conditions. The highly stress-resistant and genetically stable mutant individuals obtained from different selections were also analyzed for their cross-resistance against different stress types. The molecular analyses of the mutants were performed using diverse omic technologies such as DNA microarrays, whole genome resequencing and proteomics, and the results were compared to that of the reference strain, to understand the complex molecular basis of the stress-resistant phenotypes.

It was generally observed that the mutants selected under a particular stress condition also acquired resistance against other stress types, which contributed to their robustness. Additionally, key metabolic pathways and/or gene groups were identified for each stress resistance. An overview of our research results on various stress-resistant mutants will be provided, including the above-mentioned stress-resistant mutants, and an evolved mutant with increased chronological life span, another industrially desired characteristics.

To summarize, evolutionary engineering is a powerful strategy for industrial yeast strain development, as well as for the analysis of genetically complex properties such as stress resistance. Additionally, unlike recombinant DNA technology, it does not involve the addition of foreign genes into yeast cells. Thus, it can be viewed as a more natural procedure with a higher public acceptance for food bioprocesses.

Keywords: Evolutionary engineering, *Saccharomyces cerevisiae*, Stress resistance, Systems biology

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Postzygotic Genome Evolution in *Saccharomyces* Interspecies Hybrids

ORAL PRESENTATION ID: 121

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The *Saccharomyces* species are biologically isolated by double sterility barrier. The (alloploid) hybrid cells cannot conjugate because the *MATa/MATalpha* heterozygosity suppresses the activity of the mating-specific genes and cannot produce viable gametes (ascospores) because the (allosyndetic, homeologous) chromosomes of the (sub)genomes cannot pair (correctly) in meiosis-I. The sterility barrier can be broken down by the loss of *MAT* heterozygosity that eliminates the block of the mating programme. The restoration of the mating activity is usually due to the loss (malsegregation) of Chromosome III in one of the subgenomes. This chromosome carrying the *MAT* locus can be lost both during vegetative propagation of the hybrid cells and at meiosis upon spontaneous genome duplication. Genome duplication allows meiosis because the allotetraploid cell has two sets of chromosomes in both subgenomes. The homologous (autosyndetic) chromosomes pair within each subgenome in prophase-I and then segregate during the rest of meiosis to pass single copies of each chromosome of both subgenomes into each spore (autodiploidised allotetraploid meiosis). The resulting allodiploid spores are viable but sterile because of their *MATa/MATalpha* heterozygosity (no mating activity) and defective in meiosis (no viable ascospores are produced). Thus, genome duplication does not restore fertility; it only allows the production of sterile spore clones. However, during autodiploidised allotetraploid meiosis, Chromosome III frequently gets lost (malsegregates) in one or in the other subgenome which results in alloaneuploid spores. As these spores have only one Chromosome III, and hence only one *MAT* locus, their mating programme is active. The loss of *MAT* heterozygosity reactivates the mating-type switching mechanism as well. The vegetative descendants of the spores (monosomic for Chromosome III) can switch their mating type and then mate with each other to generate fertile allotetraploids disomic for Chromosome III. When these clones sporulate, additional chromosomes can easily be lost. Recurrent chromosome losses and occasional interactions (by allosyndetic chromosome pairing) between the subgenomes result in chimeric genomes (GARMe: Genome AutoReduction in Meiosis). Genome reduction and chimerisation also take place during vegetative (mitotic) propagation of cells (GARMi: Genome AutoReduction in Mitosis; frequently called “genome stabilisation”), although at much lower frequency. Either of the parental mitochondria and even their recombinant forms can coexist with the same hybrid nuclear genome, indicating that nucleo-mitochondrial incompatibilities do not play major roles in hybrid sterility.

Keywords: Intespecies, Hybrid, *Saccharomyces*, Genome

Development of Synthetic *Pichia pastoris* Alcohol Dehydrogenase (ADH) Promoters

ORAL PRESENTATION ID: 235

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Pichia pastoris (*Komagataella phaffii*) is a non-conventional Crabtree-positive yeast with a capability of reaching very high cell densities in a fed-batch fermentation process. It is not expected to produce ethanol at high glucose concentrations under aerobic conditions, since it is classified as a non-fermentative yeast. The AD genes involving in ethanol metabolism were identified and characterized in our previous studies. The gene responsible for the consumption of ethanol was determined as the ADH2 gene and it was highly expressed on ethanol as a sole carbon source.

In this study, the regulatory DNA regions that are responsible for the activation and repression of the ADH2 promoter of *P. pastoris* were investigated by deletion analysis. Xylanase B gene was used as a reporter to determine the promoter strength. Five different synthetic promoters were constructed by adding or deleting the regulatory sites and tested at the shake flask level by inducing with ethanol. The best synthetic promoter which had the highest activity was compared with the native ADH2 promoter at fermentor scale. One of the synthetic promoters was able to double the xylanase yield at the 5-L fermentor scale compared to the native ADH2 promoter.

Keywords: *Pichia pastoris*, ADH2 promoter, Ethanol metabolism, Recombinant protein production

Acknowledgements: This project was funded by the Scientific and Technological Research Council of Turkey (TUBITAK Grant number 215Z116) and Akdeniz University Scientific Research Projects Coordination Unit (Project number FDK-2015-691)

World of Small Molecules in Non-Conventional Yeasts

ORAL PRESENTATION ID: 200

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Non-conventional yeasts (NCY) attract increasing attention in (fermentation) industry both in industrial context (e.g. commercial value of biomass, heterologous protein expression, wide-range of substrate portfolio, etc.) as well as in fundamental science applications (different stress response compared to conventional baker's yeasts, rich molecular portfolio for stress tolerance, etc.). Following this, the number of post-genomic studies and available dedicated molecular biology toolbox is increasing. Despite the increasing interest, studies on the response of the metabolism of NCYs to different environmental stresses are at its infancy. For various stress responses (e.g. production of antioxidants, up/down regulation of various proteins), a key question is how a specific stress response interacts with central carbon metabolism to not only understand the nutrient and cofactor flow, but also for regulation, as well as energetics of growth under stress conditions.

Among the –omic studies, metabolome focuses to (quantitative) identification and quantification of low molecular weight molecules, metabolites. Typical workflow for quantitative metabolome studies involved (i) quenching to be able to take a “snapshot” of current intracellular status, (ii) extraction of intracellular content of the cell and (iii) identification and quantification of small molecules (e.g. using chromatographic separation and MS based identification) present in the intracellular fluid. Metabolome studies typically suffer from leakage to extracellular media, inefficient extraction causing over or under representation of e.g. phosphorylated compounds, peak identification in MS, (very) low amounts etc. Currently, there is little to no information on metabolome studies in non-conventional yeasts.

Following this, the aim of this study is to determine short-term metabolomic response of two non-conventional yeasts (*Yarrowia lipolytica*, *Pichia pastoris* and *Candida utilis*) upon oxidative stress (hydrogen peroxide) application to controlled fermentations at steady-state and to compare these results with the ones from conventional yeast (*S. cerevisiae*). Generally, *Yarrowia* is more resilient to oxidative stress and several amino acids in *P. pastoris* and *S. cerevisiae* respond in opposite manner. Overall, our results illustrate that NCY respond to stress in a highly different manner in cell physiology and metabolome when compared to conventional yeast.

Keywords: Non-Conventional Yeast, Metabolome, Stimulus Response Experiments.

SESSION 8: Yeast Genetic and Genomic

Erythritol Metabolism in *Yarrowia lipolytica* and Engineering Tools Derived Thereof

ORAL PRESENTATION ID: 246

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Y. lipolytica is a non-conventional yeast, well-known for its unusual metabolic properties. Based on its ability to secrete high amounts of proteins and metabolites of biotechnological interest, *Y. lipolytica* has several industrial applications, including heterologous protein synthesis or organic acid production. Under osmotic stress, the yeast produces erythritol, a four-carbon sugar alcohol. As this metabolite was found embed with sweetening properties, applications as food additive have been developed. Here, a metabolic strategy yielding to strains with increased erythritol production will be detailed together with a bioconversion process leading to erythrulose, the first intermediate of erythritol catabolism. From erythritol metabolism, we developed new efficient promoters and recipient strains that could be used for recombinant protein production and metabolic engineering. As a case study, the lipase CalB from *Candida antartica* was cloned under the control of the best-developed promoter. The lipase titer obtained in bioreactor was compared to those obtained in *P. pastoris*, another well known cell factory.

Keywords: *Yarrowia lipolytica*, Erythritol, Promoter, CalB lipase

Adaptive Evolution of Sugar Metabolism Networks in Domesticated Lineages of *Saccharomyces cerevisiae*

ORAL PRESENTATION ID: 100

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The budding yeast *Saccharomyces cerevisiae* has been used worldwide for food and beverage fermentation for thousands of years. The domesticated populations of the yeast show significantly elevated maltose utilization abilities compared with its wild lineages. In addition, the specific Milk lineage exhibits faster galactose utilization rate. The purpose of this study is to illuminate the molecular mechanisms of the improved sugar metabolism traits of domesticated yeast lineages.

We sequenced *S. cerevisiae* isolates representing different wild and domesticated lineages using the combined Illumina and the PacBio long-read sequencing strategies and performed SNP, copy number variation, structure variation, introgression and horizontal gene transfer analyses. The phenotypic consequences of the genetic changes were confirmed by gene deletion or swap experiments.

We generated high quality genome assemblies for the *S. cerevisiae* isolates compared and dissected the fine structure of the *MAL* network which usually located in subtelomeric regions of different chromosomes. We found a remarkable increase of the *MAL* genes in contents, copy numbers, structural complexities and translocation events from the wild to domesticated lineages. These polygenic changes in the *MAL* network are collectively responsible for the significant elevation of maltose metabolism in the domesticated lineages of *S. cerevisiae*.

Though *S. cerevisiae* is unable to utilize lactose which is the sole primary carbon source in milk, it is usually one of the dominant microbial species in spontaneously fermented dairy products, in which glucose and galactose released from lactose hydrolysis by other co-existing microorganisms are available for the yeast to grow. We show here that the milk lineage of *S. cerevisiae* has swapped all its structural *GAL* genes with early diverged versions through introgression and duplicated the introgressed *GAL2* gene. The rewired *GAL* network has achieved galactose-over-glucose preference switch, abolished glucose repression, and conversed from a strictly inducible to a constitutive system through polygenic changes in the regulatory components of the network. The reverse evolution of the *GAL* network confers a competitive advantage to the Milk lineage of *S. cerevisiae*, which can use galactose first immediately when it is available and minimize carbon source competition with other co-existing microorganisms usually preferring glucose.

Keywords: *Saccharomyces cerevisiae*, Domestication, Genome evolution, *MAL* network, *GAL* network

From Protein Translation to Fluxes – Quantitative Systems Biology of Yeast Stress Responses

ORAL PRESENTATION ID: 156

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Yeast is the most used microorganism in industrial biotechnology processes and often adapted as a eukaryotic model organism for aging and human disease studies. To understand these organisms better and create more efficient cell factories, we have focused on various aspects to make these cells energetically more efficient and have dedicated on creating synthetic switches to control these changes metabolically. As protein synthesis has shown to be the most energy consuming process in proliferating cells, understanding what controls protein abundances and what are the most efficient ways to regulate it represent critical questions in biology and biotechnology. Here, we have selected four industrially relevant stress conditions (high temperature, osmosis, ethanol concentration, and nitrogen limitation) and studied their gradual responses using quantitative systems biology tools. Absolute quantification of transcripts and proteins studied under 15 different stress conditions allowed us to find strong transcriptional control of protein abundances, however, substantial variability in translation rates between individual proteins. To understand the potential cause for the latter, we quantified protein turnover under several stress conditions and found only a minor contribution to the different translation efficiencies, however, contributing significantly in cellular maintenance. To achieve better predictions on metabolic fluxes, enzyme constrained genome-scale modeling approach GECKO was developed. We studied how enzyme usage varied both at the global and stress-specific levels, finding enzymes that play key roles in different pathways inside the cell at conditions of high energy demand. The study illustrates growth limitations in yeast and provides suggestions for improving cellular efficiency.

Keywords: Yeast stress, Transcriptomics, Proteomics, Protein turnover, Genome scale modeling

Acknowledgements: This project has received funding from the European Union's Horizon 2020 research and innovation program under grant agreement No 668997, and the Estonian Research Council (grant PUT1488P).

The Development of a Wide Range CRISPR-Cas9 Gene Editing System for Yeast

ORAL PRESENTATION ID: 108

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CRISPR is a revolutionary method to effectively and efficiently alter the genomic make-up of an organism. Unlike any other genetic engineering tool or technique, CRISPR is remarkably cheaper, simpler and faster to perform. In biotechnology, the best eukaryotic organism for research is yeast, due to their fast growth rate and ease of manipulation compared to multicellular organisms. Hence the aim of the study was the development of a wide range CRISPR-Cas9 system applicable in a wide variety of yeasts for easy and fast gene editing.

Three different optimized *CAS9* genes [optimized for expression in *Komagataella phaffii* (formerly known as *Pichia pastoris*), *Candida albicans* and *Homo sapiens*] were cloned into the wide range pKM180 vector. The three different *CAS9* constructs were then tested for correct expression of the Cas9 protein and the effects thereof in all the yeasts. In addition, a Ribozyme-gRNA-Ribozyme cassette was incorporated into the wide range CAS9 vector, containing the guide RNA as well as the *CAS9*. The *ADE2* gene was targeted in *Saccharomyces cerevisiae* and six other non-conventional yeast for validation of the CRISPR-Cas9 system.

Through western blot analysis it was observed that all three of the different Cas9 proteins were expressed successfully in the different yeasts, however, the *K. phaffii* optimized *CAS9* displayed several bands on the western blot membrane. It was also observed that expression of the Cas9 proteins had a negative effect on the growth of the yeast. Due to this, it was decided to only proceed with the *C. albicans* optimized *CAS9* that had the least negative effects. The system was then validated with successful disruption of the *ADE2* gene in all the selected yeasts.

This study proved that the developed of a wide range CRISPR-Cas9 system was applicable in a wide variety of different yeasts, thus allowing for rapid, cost-effective genetic manipulation of biotechnologically relevant yeast strains.

Keywords: CRISPR-CAS9, Wide-range, Yeast, Non-conventional, *Saccharomyces cerevisiae*

Maltase of *Blastobotrys adeninivorans* Displays Unusual Properties and Has Biotechnological Potential

ORAL PRESENTATION ID: 196

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Blastobotrys (Arxula) adeninivorans is an early-diverged yeast that has many physiological properties uncommon to modern yeasts like *S. cerevisiae*. On the yeast phylogenesis tree, *B. adeninivorans* resides close to *Lipomyces* species and is not very far from *Schizosaccharomyces pombe*. We consider that study of biochemistry of *B. adeninivorans* should give insight to repertoire of enzymes the yeasts had early in the evolution. The first maltase protein (AG2) of *B. adeninivorans* is characterized by us in current study. The ability of *B. adeninivorans* to metabolize a variety of maltose- and isomaltose-like sugars is evaluated revealing novel growth substrates for the yeast.

The *AG2* gene (Genes 2018, 9:354. https://doi.org/10.3390/genes9070354) was cloned from the genomic DNA of the yeast, heterologously overexpressed with a His-tag in *E. coli*, purified and assayed using common biochemical methods.

The AG2 had very high catalytic activity. It behaved like a classical maltase hydrolysing sucrose, maltose, maltotriose, maltulose, turanose and melezitose, while isomaltose and palatinose (isomaltose-like sugars) were not used as substrates. The isomaltose-like sugars and a diabetes drug acarbose served as potent competitive inhibitors of the AG2. The AG2 was capable of transglycosylation - new potentially prebiotic oligosaccharides were produced from sucrose and maltose. Surprisingly, moderate, but clearly recordable glucoamylolytic activity on polysaccharides (amylose, amylopectin and glycogen) was evaluated that is quite uncommon for yeast maltases. Based on protein sequence, the AG2 shared the highest identity (49.6%) with a maltase from a mold *Aspergillus oryzae*.

We conclude that the AG2 of *B. adeninivorans* presumably represents an ancient type of maltase protein. As this yeast grows well on many isomaltose-like sugars, it should also possess at least one isomaltase protein. Functional genomics of *B. adeninivorans* should show light on this issue.

Keywords: α-glucosidase, Transglycosylation, Yeast phylogenesis

Acknowledgements: This work was financed by Estonian Research Council grant PUT1050. Prof. V. Passoth is thanked for the *B. adeninivorans* strain.

SESSION 9: Yeast Genetic and Genomic

Construction of the Advanced Producer of Riboflavin on Whey and Lignocellulose Hydrolyzates in the Flavinogenic Yeast *Candida famata*

ORAL PRESENTATION ID: 194

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Riboflavin serves as biosynthetic precursor of flavin nucleotides FMN and FAD and is important biotechnological commodity with annual market around 250 million US dollars. Flavinogenic yeast *Candida famata* has great biosynthetic potential; however, available industrial strains are genetically very unstable. The aim of the current work was to construct highly productive genetically stable producers of riboflavin and flavin nucleotides. Gene *SEF1* coding for transcription activator was found to be the central player in providing riboflavin oversynthesis. Mutations in this gene led to inability to overproduce riboflavin. Using combination of several original approaches of classical selection, the stable riboflavin overproducing strain AF-4 has been selected. It never reverted to riboflavin non-overproducing variants as was found for industrial producer dep8. Application of metabolic engineering methods to the strain AF-4, which included insertion of the additional copies of *SEF1*, *RIB1* and *RIB7* genes (coding the first and the last structural enzymes of riboflavin synthesis), resulted in genetically stable strain of *C. famata* which accumulated during bioreactor cultivation near 16 gram of riboflavin per liter. Riboflavin synthesis was further improved due to overexpression of the engineered heterologous *Debaryomyces hansenii* genes *PRS3* and *ADE4* coding enzymes of purine biosynthesis, or the heterologous *D. hansenii* homolog of mammal gene *BCRP* coding for riboflavin excretase. It was found that riboflavin production could be also increased due to knock out of the regulatory gene of negative action *SFU1* and the structural gene coding for vacuolar ATPase *VMA1*. It was found that our advanced *C. famata* riboflavin overproducers show robust growth and efficient riboflavin synthesis in the media with lactose and xylose. Moreover, our strains accumulated high amounts of riboflavin in the media with whey and lignocellulosic bagasse hydrolyzates. Riboflavin overproducing strains of *C. famata* have been used for construction of flavin nucleotides and flavin antibiotic aminoriboflavin. Thus, the overexpression of genes *FMN1* and *FAD1* resulted in strains capable of overproduction of flavin coenzymes FMN and FAD whereas overexpression of heterologous gene *rosB* from actinomycete *Streptomyces davawensis* in FMN overproducing strain led to yeast transformants accumulating aminoriboflavin.

Keywords: Riboflavin, *Candida famata*

Insights into Phenotypic and Genetic Characteristics of the Spoilage Yeast *Zygosaccharomyces (para) bailii*

ORAL PRESENTATION ID: 190

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Food spoilage is associated with the growth of microorganisms in food and/or biochemical activities which changes food characteristics rendering it unsafe for consumption. *Zygosaccharomyces bailii* is a yeast well known for its high resistance to preservatives such as weak acids, high concentrations of sugars and ethanol. These properties make it one of the main responsible microorganisms for losses in food and beverage industries. Nevertheless, the mechanisms behind these properties are rather poorly understood resulting in difficulties to control spoilage action in many different products. Improving the knowledge of *Z. bailii* is therefore of high interest for the development of new and more efficient preservatives as well as for its potential exploitation as a cell factory. The aim of this work is the phenotypic and genetic characterization of *Z. bailii* and *Zygosaccharomyces parabailii* – a closely related species, for further quantitative trait loci (QTL) analysis.

Growth of *Z. bailii* sensu lato strains in stress conditions resembling those encountered in food, such as high concentrations of the preservative acetic acid, osmotic stress and high and low temperatures was assessed by a high-throughput tool for phenotyping on solid media. Furthermore, chromosome separation by contour-clamped homogeneous electric field (CHEF) and estimation of DNA content by flow cytometry led to the identification of the number of chromosomes and ploidy of the different strains. For QTL analysis, crossings of phenotypically different strains is required. To that end, isolation of auxotrophic mutants is ongoing in order to facilitate mating by complementation. Auxotrophic strains for ura3 and lys2 are isolated by selecting for randomly occurring mutants on synthetic media with 5-fluoroorotic acid and α-aminoadipic acid, respectively.

The strains exhibit an overall high resistance to the tested conditions with some variances. Chromosome numbers are different in some strains, which is in accordance with their hybrid origin. These data bring insights into the phenotype and genetic characteristics of *Z. bailii*. Moreover, strains with opposite phenotype are the target strains for mating and subsequent identification by QTL mapping approaches of loci associated with their resistance to stress conditions.

Keywords: *Zygosaccharomyces bailii*, Resistance, Genetic characteristics

Acknowledgements: This project has received funding from the European Union's Horizon 2020 research and innovation programme under the Marie Skłodowska-Curie grant agreement No 764927.

Improving Yeast Stress Tolerance by the Synthetic Shuffling of the PolyA Binding Protein (Pab1) Domains

ORAL PRESENTATION ID: 173

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Bio-based microbial processes are often imposing diverse stresses to the cell factories, causing impairment of titer, yield and productivity. The development of robust strains able to match industrial requirements represents therefore a pivotal challenge for the implementation of cost-effective bioprocesses. Altering master regulators of cellular networks represents one of the most suitable strategies to rewire cell metabolism towards the desirable features. We selected the poly(A) binding protein Pab1 as a target: it plays a central role in the mechanisms responsible for the fate of mRNAs and for the regulation of eukaryotic gene expression.

Pab1 is a modular protein composed of four RNA Recognition Motifs (RRM), a flexible linker rich of proline (for this reason named P domain) and a carboxy-terminal domain (C domain). Thanks to its modular structure, Pab1 regulates the fate of mRNA by simultaneously binding the mRNA poly(A) tails and by interacting with diverse proteins involved in mRNA biogenesis and decay. Here we used golden gate strategy to shuffle the Pab1 domains in either number and/or position. With the obtained plasmid library, covering 3 times the possible number of combinations, we transformed *Saccharomyces cerevisiae* wild type cells and plated them to select strains with improved robustness against different stressing agents, among which acetic acid. Plasmids harboured by these strains have been recovered and sequenced for determining the structure of Pab1 variants conferring the augmented robustness. Remarkably, most of the variants presented a higher number of RRM motives in respect to the wild type proteins.

The construction of Pab1 chimeras is offering the possibility to further study the contribution of the different parts of the protein in the assembly and clearance of stress granules, with consequences on cellular robustness. These findings pave the way for a novel approach to unlock industrially promising phenotypes through the modulation and/or the synthetic reconstruction of a post-transcriptional regulatory element.

Keywords: Stress tolerance, PolyA Binding Protein (Pab1)

Construction of the Riboflavin-Overproducing Strain of the Yeast *Komagataella pastoris* Producing the Flavin Antibiotic Aminoriboflavin

ORAL PRESENTATION ID: 210

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Among natural flavins, the antibiotic roseoflavin produced by soil actinomycete *Streptomyces davawensis*, is known. It has antibacterial activities against Gram-positive bacteria, however, it is quite toxic to mammals. At the same time, biosynthetic precursor of roseoflavin, aminoriboflavin, exhibits antibacterial properties against number of Gram-positive pathogenic bacteria being non-toxic to mammal cells.

The aim of this work was to construct yeast strains overproducing aminoriboflavin using riboflavin overproducing mutants of *Komagataella pastoris* as the parental strain.

The methods of modern molecular genetics and genetic engineering of microorganisms have been used. As the parental strain, the riboflavin-overproducing mutant of *K. pastoris* was used.

Synthetic *rosB* gene of *S. davawensis* coding for aminoriboflavin synthase with adapted to *K. phaffii* codons was used. It was cloned, along with native *K. pastoris FMN1* gene coding for riboflavin kinase, under control of strong constitutive promoters *TEF1* or *AOX1*. Constructed plasmid with *rosB* and *FMN1* genes were used for *K. pastoris* transformation. Several isolated transformants synthesized new flavin which was purified and characterized. According to chromatographic mobility and absorption spectra, the new flavin corresponded to aminoriboflavin. It is interesting to mention that the new flavin exhibited antibacterial activities against *Staphylococcus aureus* MRSA strains.

The obtained data prove the possibility to construct the producers of the active bacterial antibiotics in yeast organisms.

Keywords: Yeast, Antibiotics, Metabolic Engineering

Acknowledgement: This work was supported by bilateral Polish-Austrian (grant no. DWM.ZWB.183.218.2015.) and Ukrainian-Austrian exchange grants.

CRISPR-CAS9 Gene Editing Tools in Pathogenic and Non-Pathogenic Yeasts

ORAL PRESENTATION ID: 112

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CRISPR-Cas9 is a revolutionary method to effectively and efficiently alter the genomic make-up of an organism. This system uses a short stretch of DNA, complementary to the gene of interest, as well as the *CAS9* gene, which are introduced into targeted cells. The complementary sequence, known as the guide DNA (gDNA) sequence, is transcribed into guide RNA (gRNA) and forms a complex with the Cas9 protein. When a sequence complementary to the gRNA is found in the genome of the target cell, a single double strand break is made by the Cas9 endonuclease in this complementary sequence.

We have successfully used (with small adaptations) the CRISPR-Cas9 system developed for *Candida albicans* by the Hernday lab. We furthermore developed a similar system for *Candida auris* where the promotors and integration sites of the *C. albicans* system was replaced for optimal efficacy in *C. auris*. A wide range CRISPR-Cas9 system was also developed. Three different codon optimized CAS9 genes (optimized for *Pichia pastoris*, *Candida albicans* and *Homo sapiens*) genes was tested using the wide range expression vector pKM180. For expression of the gRNA, a Ribozyme-gRNA-Ribozyme cassette was used that was regulated by the *Yarrowia lipolytica TEF1* gene.

Using an available CRISPR-Cas9 system we deleted various genes in *Candia albicans* including *HST6, IPTI, ADE2, RTA3, SET3, WOR1* and others and we also constructed a PMA1: GFP fusion using this system. A similar system for *Candida auris* was developed and validated through the deletion of the *ADE2* gene. A wide range yeast system was also developed and this system was validated with successful disruption of the *ADE2* gene in *Arxula adeninivorans*; *Debaryomyces hansenii*; *Kluyveromyces lactis*; *Ogataea polymorpha*; *Komagataella phaffii*; *Saccharomyces cerevisiae and Yarrowia lipolytica.*

Here we show the effective use of a CRISPR-Cas9 gene editing system in *Candida albicans* as well as the use of an adapted system for application in *Candida auris*. Furthermore, a wide rage yeast CRISPR-Cas9 system was developed and validated through successful disruption of the *ADE2* gene in a variety of yeast.

Keywords: CRISPR-CAS9, Pathogenic, Non-Pathogenic, Yeast, *Candida* species

SESSION 10: Yeast General

Effect of Heat Pre-Treatment on Aminoacidic Profile in Fermentation of Synthetic Must Achieved by *Saccharomyces cerevisiae*

ORAL PRESENTATION ID: 202

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In this study, we determined the changes in aminoacidic profile of the syntethic must during anaerobic fermentation of strain *Saccharomyces cerevisiae* Fermicru AR2 No. LO122. The effect of a cell heat pre-treatment before fermentation was also investigated.

S. cerevisiae was inoculated in synthetic must, as such or subjected to a pre-treated treatment (45°C for 1 hour). Aminoacids were detected in must supernatant as follows: an Acquity HClass UPLC (Waters, Milford, MA, USA) system equipped with a photo diode array detector 2996 (Waters) was used. Compounds were separated in a Kinetex 5 um EVO C18, 150x4.6 mm (Phenomenex) column at 30°C. The mobile phase used was as follows: phase A - K_2HPO_4 20 mM pH 7.2, phase B - MetOH/ACN 50/50, A:B 80:20, flow rate 1.0 mL/min. Fluorescence detection was used at an excitation wavelength of 338 nm (for all amino acids except proline) and 262 nm (for proline). The separation and quantification of 19 amino acids and in a single run as their OPA/MCE derivatives eluted within 80 min.

Data showed that in control samples, the level of 9 amino acids was found to increase, while only of 4 increased in the must belong to pre-heated samples up to 48 h fermentation. Throughout all the trials, total amino acids consumption was greater in the pre-treated samples than in the control. According to the obtained ratio, a greater production of glycine and proline in the pre-treated sample was observed compared to the control. Within 48 h, heat pre-treated cells produced 1121% more glycine and 68% more proline than control samples.

Pre-treatment is a mild stress application causing yeast adaptation to the environment that may also lead to yeast growth increase. This research showed that heat pre-treatment increased the synthesis of proline, a stress protectant, but also the consumption of other amino acids during fermentation. These behaviour can ultimately effect wine sensory properties due to the presence of different amino acids in wine.

Keywords: *Saccharomyces cerevisiae*, Stress, Pre-treatment, Amino acid, Proline

Biocontrol Capability and Action Mechanisms of *Aureobasidium pullulans* and *Pichia guilliermondii* Against Blue and Green Moulds

ORAL PRESENTATION ID: 212

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Postharvest losses of fruits and vegetables constitute significant ratio for food industry. Today, usage of synthetic fungicides is main controlling method of postharvest pathogens. Due to strong consumer demand for chemical reduced or free foods, alternative control methods have been increasingly explored. Among various alternatives, use of antagonistic yeasts was emphasized for managing postharvest diseases. Present study aimed to clarify biocontrol potential and determine control mechanism of *A. pullulans* and *P. guilliermondii* against two postharvest pathogens (*Penicillium* (*P.*) *expansum*, *P. digitatum*) in vitro conditions.

Dual culture assay was used for preliminary screening of selected yeasts. Inhibitory effect of volatile organic compounds (VOCs), iron depletion, production of extracellular lytic enzymes, effect of yeasts on spore germination of moulds were tested to identify biocontrol mechanism of yeasts. Percent inhibition of radial growth of pathogens was also determined. Presence of protease, chitinase, gelatinase, pectinase and β-1,3 glucanase was investigated to characterize cell wall lytic enzymes in yeasts. Influence of different yeast cell concentrations (10^6 cells/mL and 10^8 cells/mL) and pH (4.5 and 6.0) on biocontrol ability of yeasts were studied. Individual and mixed yeast cultures were tested to determine the efficacy of antagonists.

Growth of pathogens was limited by both yeasts in all tested mechanisms except iron depletion. Tested two yeast strains showed almost similar inhibition ability against *P. expansum* and *P. digitatum* in dual culture assay. Development of fungal pathogens reduced by half via producing VOCs. Mixed cultures of yeasts reduced the mean germination rate of *P. expansum* and *P. digitatum* as 86% and 95%, respectively. *A. pullulans* strain restricted the radial growth of *P. expansum* and *P. digitatum* by ratio 75% and 84%, respectively. Additionally, both yeast strains showed hydrolytic enzymatic activity to degrading the cell wall of postharvest pathogens. *A. pullulans* and *P. guilliermondii* were positive for protease, gelatinase and β-1,3-glucanase activities although negative those pectinase and chitinase activities.

The results demonstrate that space-nutrition competition, production of VOCs and hydrolytic enzymes are main action mechanisms of *A. pullulans* and *P. guilliermondii* strains. Inhibition activity of these yeasts does not depend on iron competition while germination of spores significantly inhibited by mixed culture. According to results, using mixed culture and increasing the concentration of yeast cell number gave better results for controlling *P. expansum* and *P. digitatum*. This study proved that antagonistic yeasts are successful for controlling postharvest disease even though today there is no any antagonist as effective as pesticides.

Keywords: Yeast, Antagonist, Biocontrol, Postharvest, Pesticides

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Predominant Yeasts in the Sourdoughs Collected from Different Parts of Turkey

ORAL PRESENTATION ID: 211

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Sourdough is a mixture of flour and water that are spontaneously fermented with lactic acid bacteria and yeasts. In the present study, totally 8 sourdough samples produced without commercial baker's yeast addition were collected from three different bakeries at two different times in Turkey. Chemical properties of the sourdoughs were investigated and yeasts were identified by molecular methods.

Potential yeasts were grown on YPD and L-lysine agar media at 28°C for 48-72 h. Colonies those differ in color, shape or appearance were randomly picked from plates and each colony was re-streaked again onto a separate YPD agar plate by plate-streaking technique until a pure culture was obtained. All cultures were subjected to genotypic characterization by ITS region amplification of the 5.8S rRNA gene, its Restriction Fragment Length Polymorphism (RFLP) analysis and identification by sequence analysis of the D1/D2 domain of the 26S rDNA gene. Isolates were subjected to DNA extraction after lyticase (L4025, ≥200 units/mg solid, Sigma-Aldrich, MO, USA) treatment. 5.8S ITS rRNA region of the genomic DNA of the isolates were amplified using primers ITS1 (5'-TCCGTAGGTGAACCTGCGG-3') and ITS4 (5-TCCTCCGCTTATTGATATGC-3′) and PCR products showing visible bands on the agarose gel were subsequently digested using the restriction endonucleases *Hae III, Hha I* and *Hinf I*.

A total of 7 profiles were determined according to the restriction fragments. Yeasts sharing identical restriction patterns were classified into groups and representative cultures of each group were chosen for sequence analysis of the D1/D2 domains of the 26S rRNA gene. Resultant sequences were compared with nucleotide sequences of the closest relatives deposited at the database of National Center for Biotechnology Information (NCBI). Yeast strains in sourdough samples belonged to 4 genus including *Saccharomyces* spp., *Kazachstania* spp., *Pichia* spp. and *Hanseniaspora* spp. and totally 148 yeast isolates were identified at the species level (≥400 bp, 99% identity) as *Saccharomyces cerevisiae* (106), *Kazachstania bulderi* (11), *Pichia fermentans* (9), *Pichia membranifaciens* (8), *Kazachstania servazzii* (7), *Kazachstania* unispora (4) and *Hanseniaspora valbyensis* (3).

Saccharomyces cerevisiae was the most frequently isolated yeast species. Collected sourdoughs were produced without using baker's yeast. However, *Saccharomyces cerevisiae* was isolated from all of the samples and this can be related to the contamination of the bakery environment with commercial baker's yeast. Identified yeast species were same at two different sampling times in two wheat sourdough samples. On the other hand, yeast species exhibited differences at different sampling times in the other two samples.

Keywords: Wheat, Rye, Yeast, Sourdough, RFLP, 26S rRNA

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Fermented Honey and Manna Ash Products: Novel Ecological Niches of Wine Yeasts

ORAL PRESENTATION ID: 236

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The selection of novel strains of yeasts is still relevant to improve flavour of wines produced around the word. Several food niches have not been microbiologically investigated and they might represent important sources of microorganisms with technological aptitudes, e.g. in wine industry. To this purpose, two novel yeast communities associated with matrices rich in carbohydrates and characterized by low levels of water activity (a_w), such as fermented honey by-products (FHP) and "Manna" ash products (MAP) extracted from *Fraxinus angustifolia* (*Oleaceae*), were investigated. FHP contain mainly fructose and glucose, while MAP ash is mainly characterized by high concentrations of mannitol, fructose and mannotriose. The values of a_w of both matrices is around 0.5-0.6. Yeasts were isolated, subjected to the genotypic identification and then technologically characterized to evaluate their oenological potential.

The species *Lachancea fermentati*, *Pichia anomala*, *Pichia kudriavzevii*, *Saccharomyces cerevisiae*, *Wickerhamomyces anomalus*, *Zygosaccharomyces bailii* and *Zygosaccharomyces rouxii* were genetically identified from FHP samples. During the spontaneous alcoholic fermentation, the dominating species were *S. cerevisiae*, *Z. bailii* and *Z. rouxii* whose feed conversion ratio of sugars into ethanol was about 53%. On the other hand, MAP was characterized by the presence of *Candida aaseri*, *Candida lactis-condensi*, *Citeromyces matritensis*, *Lachancea thermotolerans*, *Saccharomyces cerevisiae* and *Zygosaccharomyces bailii*. Interestingly, both matrices showed the presence of *S. cerevisiae* at consistent levels and a high number of L. thermotolerans strains were isolated from MAP. Both species have interesting eonological potential.

Six strains of *S. cerevisiae* were tested as starters to ferment grape must from Grillo, Catarratto and Chardonnay cultivars at industrial winery-scale over two consecutive years. Interestingly, four strains isolated from FHP showed a fructophilic potential and the experimental wines were characterized by an intense floral flavour. Among non-*Saccharomyces* yeasts, *L. thermotolerans* strains showed resistance to ethanol up to 12-13 % (v/v) and ability to ferment grape must with a feed conversion ratio of sugars into ethanol of about 45-55%. Eonological potential was showed by *C. lactis-condensi* and *C. aaseri* since a high content of glycerol was produced at the end of alcoholic fermentation and a fructophilic aptitude was found.

In conclusions, the present research provided novel microbiological and physicochemical insights on the alcoholic fermentation conducted by novel starters belonging to *S. cerevisiae* and *L. thermotolerans* species and, for the first time, the species *C. lactis-condensi* and *C. aaseri* were found to be of relevance for wine application.

Keywords*:* Wine production, Fermented Honey

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SESSION 11: Yeasts as Sources of Ingredients and Additives

Metabolic Engineering of *Yarrowia lipolytica* for Production of Tailor-Made Lipid Compounds

ORAL PRESENTATION ID: 137

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The work is focused on production of various lipid compounds by genetically engineered oleaginous *Yarrowia lipolytica* strains.

Genes coding biosynthesis of special lipid structures in plants and insects were characterized and their sequences were optimized before their transformation to *Y. lipolytica*. Insertion cassettes equipped with special promoters were used for transformation of these genes to the yeast. *Y. lipolytica* served as a host for introduction of following genes: a) *FAE1* gene from field pennycress (*Thlaspi arvense*) coding for fatty acid elongation involved into biosynthesis of erucic acid (22:1ω9, EA), b) *EaDAcT* gene from *Euonymus europaeus* coding for diacylglycerol acetyltransferase catalyzing formation of 3-acetyl 1,2-diacylglycerol, c) *MsexD2* and *MsexD3* genes from tobacco hornworm moth (*Manduca sexta*) which encode two fatty acid desaturases, and d) *BlucFar1* and *BlapFar4* genes from two bumblebee species (*Bombus lucorum* and *Bombus lapidarius*) which encode two fatty acid reductases.

FAE1 from *T. arvense* overexpression in *Y. lipolytica* mutant strain possessing *fad2* deletion led to accumulation of almost 10% of EA in cells lipids, exclusively in triacylglycerols. Other elongation products of oleic acid (C20:1 and C24:1) were observed as well. Moreover, percentage amounts of C20:0, C22:0, and C24:0 fatty acids increased at least two-fold in the presence of *FAE1* gene. Overexpression of *EeDAcT* in *Y. lipolytica* caused formation of 3-acetyl 1,2-diacylglycerol (acTAG) in intracellular lipids. Interestingly, acTAG was accumulated in all strains regardless to their possibility to either form or not form storage lipid particles. Expression of *MsexD2* and *MsexD3* genes resulted in biosynthesis of mainly C16:1Δ^{11} fatty acid (11-14% of total fatty acids). Production of other new fatty acids C14:1Δ^{11} and C18:1Δ^{13} were also observed in trace amounts. Media supplementation with precursors C16:1Δ^{11} and C16:2$\Delta^{10,12}$ led not only to formation of C16:2$\Delta^{10,12}$ and C16:3$\Delta^{10,12,14}$, but also new C18 fatty acids with two and three double bonds were detected. Finally, accumulation of long chain alcohols in *Y. lipolytica* expressing bumblebee reductases was confirmed. However, the strain with *BlucFar1* gene formed C18, C20, C22, C24 fatty alcohols, while C16 alcohols were observed in strain with *BlapFar4* gene.

Oleaginous yeast *Y. lipolytica* with rational metabolic engineered strategies showed a great potential for production of various tailor-made lipid structures with their several applications in pharmaceutical, nutritional and industrial fields.

Keywords: *Yarrowia*, Metabolic engineering, Lipid compounds

Acknowledgements: The work was supported by grants VEGA 1/0323/19 (Slovak Ministry of Education, Science, Research and Sport) and APVV-17-0262 (Slovak Research and Development Agency).

Conversion of Sugars and Phenolic Compounds to Secreted Surfactants by *Rhodotorula* Yeasts

ORAL PRESENTATION ID: 181

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We recently showed that certain species of oleaginous yeasts in the genus *Rhodotorula* grown on glucose secrete a new class of amphiphilic glycolipids called polyol esters of fatty acids (PEFA), which are a new class of biosurfactants, and simultaneously accumulate intracellular triacylglycerols (iTAG). The objective of this study was to determine what other carbon sources could be converted to iTAG and secreted PEFA.

Strains of several PEFA-secreting *Rhodotorula* species from the Phaff Yeast Culture Collection at UC Davis were cultivated in yeast nitrogen base supplemented with sugars or phenolic compounds to test for ability to assimilate these compounds. Positive combinations of yeasts and carbon sources were then grown in liquid media, and iTAG and secreted PEFA were extracted and quantified. One yeast/carbon source combination was selected for cultivation at 4-liter scale in a bioreactor, and iTAG and PEFA were quantified.

Growth of most PEFA-secreting yeast species was poor or undetectable in yeast nitrogen base supplemented with xylose, arabinose, rhamnose, galacturonic acid, or most phenolic compounds. Growth and PEFA production were weak to moderate on acid forms of phenolic compounds (vanillic acid, p-hydroxybenzoic acid (PHBA) or coumaric acid), glycerol or molasses. Growth and PEFA production were strong on glucose or sucrose. Yields of iTAG and PEFA for *Rhodotorula* aff. *paludigena* UCDFST 81-84 grown at 4-liter scale in a bioreactor with 100 g/L sucrose for 6 days yielded 18.1 g/L TAG plus 21.2 g/L PEFA.

Keywords: Glycolipid, Lipid

Yeast *Cornucopia* – Bioinspired Gold Nanoparticles Synthesis

ORAL PRESENTATION ID: 159

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Synthesis of nanoparticles (NPs) and their incorporation in materials are amongst the most studied topics in chemistry, physics and material science. Gold NPs have applications in medicine due to their antibacterial and anticancer activities, in biomedical imaging and diagnostic test. Despite chemical synthesis of NPs are well characterized and controlled, they rely on the utilization of harsh chemical conditions and organic solvent and generate toxic residues. Therefore, greener and more sustainable alternative methods for NPs synthesis have been developed recently. These methods use microorganisms, mainly yeast or yeast cell extract. NPs synthesis with culture supernatants are most of the time the preferred method since it facilitates the purification scheme for the recovery of the NPs. Extraction of NPs, formed within the cells or cell-wall, is laborious, time-consuming and are not cast effective. The bioactivities of NPs, namely antimicrobial and anticancer, are known to be related to NPs shape, size and size distribution.

Here, we proposed the method for a green synthesis of gold nanoparticles by purified yeast metabolite; melanin. This brown pigment, isolated from *Yarrowia lipolytica* strain, has been characterized as pyomelanin and its synthesis was found to occur by the so-called HGA-melanin pathway. The purified pyomelanin was found embedded with antioxidant properties. It was also characterized as non-cytotoxic toward two mammalian cell lines, namely the mouse fibroblast and human keratinocytes. When blended with different commercial sunscreens, the purified pyomelanin increased significantly the sun protection factor (SPF) value, highlighting its potential utilization as UV-filter in cosmetic preparations. Consequently, this metabolite was further used for green synthesis of gold nanoparticles. We evaluate the potential to manipulate key parameters to control the monodispersity, size and shape of NPs. Therefore, the effect of pH, temperature, substrate concentration, time and addition of stabilizing agent on the synthesis of gold nanoparticles were investigated. The physico-chemical characterizations of nanoparticles, with size distribution (DLS), UV-Vis spectroscopy, Transmission Electron Microscopy (TEM) and measurement of the NPs stabilization, were employed. A profile of the bioactivity of these nanoparticles compared to purified melanin, was assessed with cell lines. The absence of cytotoxicity of the AuNPs toward fibroblasts and osteosarcoma cells was evidenced. Moreover, they had negligible effect on cell migration and on cell morphology assessed with Scanning Electron Microscopy (SEM). These results suggest that the bio-synthesized AuNPs could have an application in cancer therapy as a drug delivery system as well as a diagnostic tool in medicine.

Keywords: Pyomelanin, *Yarrowia lipolytica*, HGA-melanin pathway, Photoprotection, Gold nanoparticle, Cytotoxicity, Mammalian cell line

Acknowledgements: Imen Ben Tahar was supported by a Post doc grant from WBI (Wallonie-Brussels Interantional), bourse d'excellence IN WBI.

Biotechnological Applications of the Yeast *Yarrowia lipolytica*

ORAL PRESENTATION ID: 168

***Zbigniew Lazar*, Magdalena Rakicka-Pustułka, Ludwika Tomaszewska-Hetman, Piotr Juszczyk, Piotr Hapeta, Anita Rywińska, Waldemar Rymowicz**

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The oleaginous yeast *Yarrowia lipolytica* has an amazing biotechnological potential. These yeasts use only a small number of sugars (glucose, fructose, mannose) or glycerol as substrates, but they are mostly known for their abilities to utilize hydrophobic substrates such as lipids and hydrocarbons. Nowadays, due to the constant, undesirable competition between food and biotechnological industry for the substrate, *Y. lipolytica* offers the opportunity to use wastes materials as carbon sources (raw glycerol, waste fats, olive mill wastewater). Furthermore, using genetic engineering, new transformants of *Y. lipolytica* with increased spectrum of available substrates were constructed. The new available carbon sources include but are not limited to lignocellulosic raw materials. Given the sacrificial possibility of using cheap and renewable raw materials, the processes with *Y. lipolytica* have huge potential to become cheap and profitable.

From the 90s of the last century, efficient processes of bioremediation of hydrocarbon contaminated soil as well as organic acids biosynthesis (citric, isocitric, pyruvic, α-ketoglutaric) were successfully developed using *Y. lipolytica*. The studies include optimization of culture conditions and utilization of cheap raw materials, such as raw glycerol or glucose hydrol. Furthermore, it turned out that wild strains of *Y. lipolytica* are very efficient polyhydroxy alcohols (erythritol, mannitol) producers. These compounds are widely used as low or even non-caloric sweeteners. Among many strains, the best sweeteners' producers were selected and very efficient biotechnological processes were developed. Moreover, metabolic pathway of erythritol biosynthesis was elucidated, and using genetic engineering, erythritol biosynthesis was improved.

Additionally, increasing the spectrum of substrates used for lipid biosynthesis by *Y. lipolytica* as well as the process of improving lipid biosynthesis itself is another important research direction in our Department and part of our international collaboration. An important aspect of research related to the production of fodder yeast was the enrichment of yeast biomass with selenomethionine and selenocysteine. Currently, the potential of *Y. lipolytica* to produce kynurenic acid is elucidated. Moreover, understanding the regulatory mechanisms or sugars and glycerol utilization, which is currently thoroughly analyzed, will allow us to develop strictly controllable and due to that, more efficient biotechnological processes using these fascinating micro-factories.

Keywords: Ketoacids, Poliols, SCO, SCP, *Yarrowia lipolytica*

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SESSION 12: Yeasts as Sources of Ingredients and Additives

Synthetic Strategies for Production of Aromatic Molecules in *Kluyveromyces marxianus*

ORAL PRESENTATION ID: 188

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Phenylpropanoids are a broad class of secondary metabolites that are synthesised from aromatic amino acids, and many have useful bioactive properties. Examples are the anti-oxidant resveratrol and the flavour, naringenin. Although yeast do not naturally synthesise phenylpropanoids, they do produce the precursor aromatic amino acids and, with the addition of heterologous enzymes, can be engineered to produce specific molecules. Generally, however, yields in *Saccharomyces cerevisiae* are too low to be commercially viable and alternative hosts are needed. The yeast *Kluyveromyces marxianus* is attractive because of a natural high flux through the pentose phosphate pathway which is the source of building blocks for aromatic amino acids. Phenylalanine, tyrosine and tryptophan are synthesised via the shikimate pathway, which is subject to feedback regulation at several steps. We have taken a dual approach to engineer *K. marxianus* strains to overproduce these aromatic amino acids. The rational approach involves removing negative feedback regulation through allelic replacement with mutated genes, and the model-guided strategy involves use of a genome-scale metabolic model to predict beneficial modifications. To facilitate pathway engineering, we developed an *in vitro* combinatorial assembly platform using the YTK Golden Gate standard as well as engineered host strains to allow straightforward mutation and integration of novel genes and clusters. In this way, we were able to significantly improve flux through the shikimate pathway, thereby generating improved supplies of phenylalanine for later synthesis of phenylpropanoid molecules. This presentation will cover our strategies and latest results in this area.

Keywords: Industrial biotechnology, Non-conventional yeasts, Synthetic Biology

Acknowledgements: The CHASSY project has received funding from the European Union's Horizon 2020 Framework Programme for Research and Innovation - Grant Agreement No. 720824

Biofuels, Feed and Food Production from Lignocellulose Using Oleaginous Yeasts

ORAL PRESENTATION ID: 145

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Production of vegetable oils has in many cases been accompanied with monocultures, land use changes, or rain forest clearings. Microbial lipids produced from lignocellulose and crude glycerol (side product of biodiesel production) can serve as sustainable alternatives to vegetable oils. Our projects aim to understand the physiology of microbial lipid production, optimise the production and establish novel applications of microbial lipid compounds. However, methods for controlled cultivation, analysis and quantification of lipid compounds and genetic manipulation of oleaginous yeasts are still underdeveloped.

We have established methods for fermentation, intracellular lipid quantification, carotenoid extraction and analyses. We are also establishing methods for genome analyses and genetic manipulation of oleaginous yeasts.

System analyses demonstrated that producing biodiesel from lignocellulose with the help of oleaginous yeasts can reduce fossil fuel consumption and the release of greenhouse gases. Some oleaginous yeasts are producing several carotenoids and extracellular biosurfactants, which can have a high value in a variety of applications. Utilising yeast oil as component in fish feed did not have any negative effects on the fish. To further check the safety of using oleaginous yeasts for feed and food purposes, we are establishing methods for toxicological analyses of yeast lipids and the whole biomass.

Based on the established methods and our results, we now have tools for exploring the full biotechnological potential of the highly diverse oleaginous yeasts and to understand their physiology, which is also of interest for fundamental science.

Keywords: Oleaginous yeasts, Lignocellulose, Biofuels, Food, Chemicals

Acknowledgements: Our research was supported by The Swedish Research Council for Environment, Agricultural Sciences and Spatial Planning (Formas), Grant Numbers 213-2013-80 and 2018-01877.

A Novel Chimeric Bioadhessive Protein Production in *Pichia pastoris* for Biomedical Applications

ORAL PRESENTATION ID: 251

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Natural bioadhessive proteins able to attach to the wet surfaces have received increased attention for their potential applications in drug delivery, tissue engineering and surgery. These proteins are biocompatible, biodegradable, non toxic and elicit only minimal immune responses in humans. As purifying natural adhesive proteins is not feasible, recombinant production of these adhesive proteins has suggested to make new adhesive biomaterials.

To designe biomimetic amyloid-based chimeric adhesive protein, we fused mussel foot protein 5 of *Mytilus californianus* and GvpA protein, the major subunit of *Anabaena flos-aquae* gas vesicle in *P. pastoris*. Various genetical and biochemical approaches were applied to enhance its expression followed by purification and in vitro post-translational modification using Ni-resin and tyrosinase respectively. Purified chimeric protein was evaluated with SDS-PAGE, Western blotting, and MALDI–TOF mass spectrometry. The general amyloid features of all adhesive fibers were detected with a Congo Red assay. Dopa residues in modified proteins were qualitatively detected by Nitro Blue Tetrazolium (NBT) staining and quantitatively analysed with acid-borate difference spectrum (ABDS) analysis.

Here, we established the first recombinant expression of strong and multi-functional chimeric adhesive protein. This hybrid molecular material is able to have function in wet condition while self-assembled into higher-order structures, in which, according to molecular dynamics simulations, disordered adhesive Mfp domains are exposed on the exterior of amyloid cores formed by GvpA.

We conclude that that introduction of the GvpA as a cross-linker reacted with the function of the Mfp5 protein and improve its stability/toughness of the adhesion. Altogether, we conclude the chimeric protein is suitable to create uniform multi-component self-assembling materials for biomedical applications.

Keywords: Chimeric protein, Bioadhessive, *P. pastoris*, Mfp5, GvpA

SESSION 13: Yeasts in Health

Towards Explaining the Metabolome of a Yeast Cell

ORAL PRESENTATION ID: 245

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The prediction of cellular metabolism represents a key problem in explaining phenotype from genotype. Indeed, predicting metabolism from the underlying gene expression data has so far proven difficult. This is, as enzymes and metabolites are in a many-to-many relationship, that are not captured with simple statistical methods - one-2-one correlations are not sufficient to predict metabolite levels from enzyme proteomes. We here present evidence that a new generation of analytical and computational methods is changing this situation and allow to dissect the interdependency of proteome and metabolome on a system-wide scale, as well as enabled the prediction of metabolism. In a collaboration with SCIEX we have developed ScanningSWATH, a method that allows the measurements of thousands of precise proteomes at high throughput and low cost. We show that the systematically recording of yeast proteomes enables to train machine learning algorithms over the topology of the metabolic network and to predict metabolite concentrations from the gene expression data. Furthermore, we will show examples, how the interpretation of the Machine-Learning models generates new biological information and testable hypotheses that concern the regulation of metabolism.

Keywords: Metabolome of a yeast cell

The Yeast Transporter Pdr5 Confers Resistance to the Antifungal Cytochalasin of *Xylaria* sp. BCC 1067

ORAL PRESENTATION ID: 175

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ATP-binding cassette (ABC) transporters are transmembrane efflux transporters involved in a variety of activities, including cellular protective mechanisms by export drugs or toxins from cells. Expression of ABC transporters such as Pleiotropic Drug Resistance 5 (Pdr5) increase resistance to the currently available antifungal drugs, demanding new agents to combat fungal infections. In this study, we identified an antifungal compound from *Xylaria* sp. BCC1067 crude extract and elucidated its structure. Moreover, we determined synergistic effect of this compound to enhance antifungal activity of azole drugs.

Antifungal compound was isolated by column chromatography and elucidated structure by NMR. *Saccharomyces cerevisiae* strains including deletion and overexpression ABC transporter were used. Minimal inhibitory concentration (MIC) assay and checkerboard assay were performed in order to examine antifungal activity and synergistic effect. Gene expression analysis and various fluorescent probes, including Phalloidin-FITC, Rhodamine 6G, 2',7'-Dichlorodihydrofluorescein diacetate, and Propidium iodide, were used to examine mechanism of action.

Antifungal compound was isolated as major component presenting in crude extract of *Xylaria* sp. BCC1067. It was identified as a cytochalasin showing low MIC_{50} and poor survival in the Δ*pdr5* strain suggesting substrate of Pdr5. In order to reduced accumulation of compound, *PDR5* gene was rapidly induced and Pdr5 transporter was active. In the Δ*pdr5* strain, cytochalasin caused actin aggregation and elevated cellular ROS level. Overexpressing *PDR5* gene enabled yeast cell increased resistance to cytochalasin and protect cell from aggregation of actin. Synergy was only observed in crude extract of *Xylaria* sp. BCC1067 combination with ketoconazole, while and additive effects in combination with other azoles including ketoconazole, clotrimazole, fluconazole, and itraconazole. Cytochalasin was selectively synergy with ketoconazole, clotrimazole, fluconazole, and itraconazole.

This study reported for the first time antifungal activity of cytochalasin that targets actin cytoskeleton mediating cell death.

Keywords: Cytochalasin, Drug resistance, Natural antifungal, Pdr5, *S. cerevisiae*, *Xylaria*

Acknowledgements: National research council of Thailand and King Mongkut's university of technology thonburi.

Arachidonic Acid Increases Expression of CDR1 in *C. albicans*, but Inhibits Efflux Activity

ORAL PRESENTATION ID: 114

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Candida albicans is a commensal of humans that can cause opportunistic infections with high morbidity and mortality. This polymorphic yeast produces antifungal resistant biofilms. One mechanism associated with this increased resistance to antifungal agents, such as fluconazole, is the overexpression of ABC efflux pumps such as Cdr1p. The addition of polyunsaturated fatty acids, such as arachidonic acid, to azoles, has been shown to increase azole susceptibility of *C. albicans* biofilms significantly. However, the underlining mechanism is not fully known. Therefore, it was necessary to investigate the influence of arachidonic acid on Cdr1p and ascertain its role in increasing azole susceptibility.

Transcriptome analyses of biofilms exposed to sub-inhibitory concentrations of arachidonic acid alone, fluconazole alone or a combination of fluconazole and arachidonic acid indicated a dose-dependent, significant increase in *CDR1* expression during early biofilm formation in the presence of arachidonic acid. The efflux activity of these proteins, measured by Rhodamine 6G efflux assay, was severely diminished in the presence of arachidonic acid, even with the addition of fluconazole. Also, in fluconazole-induced tolerant strains, tolerance was reversed by the addition of arachidonic acid. This phenomenon may be associated with a disruption in membrane/lipid raft organisation and subsequent delocalisation of Cdr1p to the cytoplasm in the presence of arachidonic acid, as visualised using *CDR1-GFP* tagged cells.

The presence of arachidonic acid can interfere with the fluconazole resistance mediated by Cdr1p, and it highlights the importance of the cell membrane in the function of membrane-associated proteins such as drug efflux pumps. Besides, this is also a reminder that the differential expression of genes does not always correspond to differences in functionality.

Keywords: *Candida albicans*, Arachidonic acid, Drug efflux pumps

Trk Transporters Mediate Potassium Uptake and Contribute to Cell pH Homeostasis and Fitness of Pathogenic *Candida* Species

ORAL PRESENTATION ID: 102

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The regulation of ion and pH homeostases is an essential process critical for cell viability. The maintenance of high intracellular concentrations of potassium and neutral pH is important for a variety of cellular functions including cell volume, DNA integrity, protein modification and trafficking. It is becoming increasingly evident that the coordination between primary H^+-ATPases and transport systems involved in the influx and efflux of potassium allows this pH maintenance to occur. Genes encoding three types of potassium uptake systems have been found in yeast genomes (Trk1 uniporters, Hak1 proton-K^+ symporters, Acu1 ATPases). Heterologous expression of these transporters in *S. cerevisiae* lacking its own potassium uptake system revealed that the activity of Trk transporters is crucial for the control of membrane potential, intracellular pH and cell fitness. Deletion of *TRK* genes results in an increase of membrane potential and decrease of intracellular pH in both osmotolerant and pathogenic yeast species. In *C. glabrata*, it results also in a change of cell surface properties, decreased virulence and pathogenicity. Taken together, our results find the Trk-type potassium uptake systems in *Candida* cells to be a promising target in the search for their specific inhibitors and in developing new antifungal drugs.

Keywords: Potassium uptake, Intracellular pH, Membrane potential, Virulence

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POSTER PRESENTATIONS

Yeasts in Fermented Foods and Beverages

From Strain to Product

POSTER PRESENTATION ID: 118

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Yeast is unique in its many applications and uses. It can be used as a nutritional additive, it can leaven dough and ferment foodstuffs, it can make biofuels, it can be used to make heterologous proteins and much, much more. University groups all over the world are finding new ways to harness the power of yeast every year, either by isolation from interesting places, directed evolution, mutagenesis, or genetic modification.

But, when a new strain has been found that has great potential in a new application, how can it be made into a commercial product? Here we show that the production process itself poses different challenges to those in the applications. Large-scale production, processing, packaging, storage and transport create large fluctuations in nutrient availability, inhibitor concentrations, osmotic pressure, temperature and water activity, as evidenced by substrate and process analyses. The yeast needs to be able to withstand all these fluctuations during the process as well as be able to cope with the often completely different conditions once it reaches the application.

AB Biotek, a division of AB mauri, specializes in bringing to market new yeast strains by using scaled down versions of the commercial processes with a high level of flexibility to try out different adjustments as well as close collaborations with strain development laboratories. Data are presented that show the improvements that can be made with proper protocol development. This way every opportunity for yeast to make the world a better place gets the best possible chance of coming to life.

Keywords: Strains, Commercialisation, Production, Stress

Biodiversity among Non-*Saccharomyces* Wild Strains as a Tool for the Selection of New Starter Cultures for Winemaking

POSTER PRESENTATION ID: 133

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The widespread use of inoculated fermentation with commercial *Saccharomyces cerevisiae* strains has determined an uniformity in wine organoleptic characteristics. Biotechnological approaches, principally based on selection of new starter cultures possessing specific properties, can satisfy the winemakers request for product differentiation. In this context, non-*Saccharomyces* yeasts deserve special attention. Although in the past these yeasts were considered as undesirable agents, actually they have been re-evaluated as their ability to produce hydrolytic enzymes and other metabolites of oenological relevance allows to improve wine quality. This study aims at screening of wild non-*Saccharomyces* strains suitable to be used as mixed starter to improve wine characteristics.

Non-*Saccharomyces* wild yeasts, belonging to different species, such as *Hanseniaspora guilliermondii, H. osmophila, Metschnikowia pulcherrima, Torulaspora delbrueckii, Saccharomycodes ludwigii*, were chosen and tested for parameters of enological interest, such as the growth in varying concentrations of ethanol and total SO_2, production of extracellular hydrolytic enzymes, such as β-glucosidase. The strains showing the best combination of parameters were chosen and tested in microvinification as mixed starter cultures in combination with a *S. cerevisiae* commercial starter, testing different inoculum modalities. In particular, the selected non-*Saccharomyces* strains were tested in mixed cultures as free and microencapsulated cells. The fermentative process was monitored by evaluating the sugar consumption during the time and by microbiological monitoring of fermentative process in order to evaluate the persistence of non-*Saccharomyces* strains during the process. The experimental wines were analyzed for the content of ethanol, volatile acidity and secondary compounds affecting wine aroma, such as esters and higher alcohols.

The obtained results confirmed the high variability among different non-*Saccharomyces* yeasts and the influence of inoculum modality on strains fermentative performance as a consequence of interaction mechanisms between strains included in mixed starter culture.

The screening of strain biodiversity and pointing-out of fermentation protocol represent useful tools to satisfy the current market trend for new style wines.

Keywords: non-*Saccharomyces* wild strains, Mixed starter cultures

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"Vino Cotto": A Precious Reservoir of Microbial Diversity

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"Vino cotto" is a wine produced in Central Italy (Abruzzo and Marche regions) according to traditional procedures involving a prolonged fermentation of cooked grape must. White or red grapes could be used for its production (Montepulciano d'Abruzzo, Trebbiano, Passerina and Moscato cultivars). Previous studies have been mostly focused on the technological aspects but very little is known on the microbiota of this product. Therefore, the aim of this work was to study the physico-chemical composition and microbial characterization of 7 samples of "Vino cotto" produced and stored in different old barrels (years 1890, 1920, 1926 and 2008).

Viable yeasts and bacteria were identified sequencing the D1/D2 domain of 26S rRNA gene and 16S rRNA gene, respectively. Strains differentiation was performed by RAPD-PCR technique. Total bacterial and eukaryotic population were also studied through High Throughput Sequencing (HTS). Volatile compounds and physico-chemical characteristics of wines were performed.

Viable yeasts identification resulted in the presence of 60% of *Candida lactis-condensi* and 30% of *Hanseniaspora uvarum*. Other yeasts belonged to *Hanseniaspora guilliermondii*, *Saccharomyces cerevisiae* and *Metschnikowia* sp. Bacterial population was characterized by the presence of lactic acid bacteria (LAB) and acetic acid bacteria (AAB): 36% of strains belonged to *Lactobacillus plantarum*, 15% to *Pediococcus pentosaceus* and 49% to *Gluconobacter oxydans*. AAB were found in older barrels and LAB in 2008s barrel. RAPD-PCR analysis highlighted a high interspecific biodiversity. HTS allowed to obtain a higher diversity of total microbial population present.

This study led to a better understanding of the microbial diversity of this traditional product. "Vino cotto" represents a wide genetic source of diverse species and strains suitable for biotechnological applications. Further studies on physiological and genetic characteristics of microbiota could contribute to a better understanding of microbial ecology and to better discover species and strains which could improve the quality of this old traditional product to maintain a strong link with the territory.

Keywords: "Vino Cotto", Yeasts, Bacteria, Cooked grape must, Volatile profile

Influence of Natural *Saccharomyces cerevisiae* Strains on Thiols Content of Pecorino Wine

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One of the frontier research in wine microbiology and winemaking industry is the preservation of terroir characteristic of each wine. This led to an increase of studies focusing on autochthonous yeasts selection which might contribute to the oenological features of a specific region. Selected strains usually produce wine with a specific aromatic fingerprint. Abruzzo region (Central Italy) is vacated to the production of red and white wines such as Pecorino. The aim of this study was to determine the impact of 10 *Saccharomyces cerevisiae* strains on varietal thiol production in Pecorino wines. These compounds are not present in must but are synthesized and released by *S. cerevisiae* during alcoholic fermentation. Varietal thiols such as 4- mercapto-4-methylpentan-2-one (4MMP), 3-mercaptohexanol (3MH) and 3- mercaptohexyl acetate (3MHA) impart fruity aroma to wines.

Ten strains were inoculated in pasteurized Pecorino must at a final concentration of 6 Log cfu/mL. Reverse transcription (RT)-qPCR of main genes involved in thiols production (*cys*3, *cys*4, *MET*17 and *IRC*7) was performed. Thiols were detected by gas chromatography coupled to mass spectrometry (GC-MS). The strain showing the highest production of thiols was used as starter culture for Pecorino wine production in a local winery during the vintage 2018.

In Pecorino must 4MMP and 3MH were produced by almost all strains with values ranging from 22 ng/L to 122 ng/L for 3MH and from 25 ng/L to 211 ng/L for 4MMP. Interestingly, strains showing the highest fold changes of tested genes released the highest concentration of thiols in wine. Cellar vinification confirmed the ability of selected strain to enhance thiols content in Pecorino wines.

Thiols production is an important trait for starter cultures selection for Pecorino wine. Obtained data underlined the relation between gene expression and thiol production. This approach could represent a promising strategy for wine yeast selection.

Keywords: Thiols, Pecorino wine, Gene expression, Yeast selection, *Saccharomyces cerevisiae*

Production of High-purity Galactooligosaccharide (GOS) by Removal of Glucose and Lactose from GOS Syrup via Yeast-based Selective Fermentation

POSTER PRESENTATION ID: 148

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Galactooligosaccharides (GOSs) are nondigestible (prebiotic) polymers, which have a beneficial effect on human health by promoting the growth of probiotic bacteria in the large intestine. GOS molecules are β-linked oligosaccharides composed of galactose, with glucose or galactose at the reducing end. Vivinal GOS is produced by the conversion of whey lactose with *Bacillus circulans* β-galactosidase. It is a syrup containing 75% dry material including the following components: GOS 59%, lactose 19%, glucose 21% and galactose 1%. Our aim was to develop a yeast-based selective fermentation process for reduction or complete removal of lactose and glucose from Vivinal GOS without decreasing its GOS content.

It is a general rule that β-galactosidases are inhibited by glucose; therefore, we developed a two-step fermentation system when glucose was used up from GOS syrup first, followed by metabolism of lactose.

In the first step glucose content of culture media containing 10 or 15% Vivinal GOS was fermented by *Pichia jadinii*. Strains belonging to three different *Kluyveromyces* species (*K. lactis*, *K. marxianus* and *K. nonfermentans*), were tested in the second fermentation step.

Glucose content of GOS culture media was completely removed by *Pichia jadinii* within 48 hours. In the second step *K. lactis* was most active in the consumption of lactose, followed by *K. marxianus*; *K. nonfermentan*s was active but slow. Ethanol contents of culture broths were almost completely consumed by the end of fermentation. Concentrations of >DP2 fractions did not change considerably during 48 hours of fermentation but decrease in their concentrations was observed during prolonged fermentation periods. This was probably the consequence of partial cell lysis and activity of the released β-galactosidases.

Selected robust food-grade yeast strains proved to be suitable for removal of glucose and lactose from GOS syrup without degradation of the valuable highly polymerized compounds. Separated yeast biomass could be valorised as dried fodder yeast, yeast extract and bioemulsifier.

Keywords: GOS-purification, Yeast fermentation

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Development of Amino Acid Analogue-resistant Sake Yeast for Commercial Scale Sake Brewing

POSTER PRESENTATION ID: 150

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Sugars, organic acids and amino acids are the major components that contribute to the taste of sake. Sugars are sweet, organic acids are sour, amino acids are sweet, bitter and umami. By changing the balance of sugars and organic acids, it is possible to make sake of various tastes. On the other hand, too many amino acids are often regarded as miscellaneous tastes. Focusing on the individual amino acids, some amino acids are tasty and some amino acids have physiological functions. If only such amino acids can be increased to sake mash, differentiated sake and sake lees can be produced.

In order to achieve this purpose, we utilized the sake yeast strains with high amino acids production. Various amino acid-analogue resistant strains were obtained and evaluated in small scale sake brewing tests. As a result, proline high producing strains were frequently found among the strains showing resistance to the proline toxic analogue azetidine-2-carboxylic acid (AZC). Many mutant strains were confirmed to have mutations in *PRO1* encoding γ-glutamyl kinase, which is a key enzyme in proline biosynthesis in *S. cerevisiae*. Such mutant strains have been reported to be desensitized to proline feedback inhibition of γ-glutamyl kinase. In addition, the *PRO1* homozygous mutants had better growth on the AZC-containing medium, and higher production of proline in the small scale sake brewing test than the *PRO1* heterozygous mutants. Many AZC resistant strains produced more proline and less succinic acid than the parental strain. The taste of proline is sweet, and the taste of succinic acid is sour and umami. So, in order to improve the taste of the light-bodied sake, we performed a small scale sake brewing test using one of the AZC resistant strain. As a result, by using the mutant strain, alcohol content and amino acid level were almost the same as the parental strain, and the mutant strain had lower acidity than the parental strain. In addition, sensory evaluation revealed that the sake brewed using the AZC resistant strain had a less sour taste.

Similar results were obtained in our brewery, indicating that the strain is industrially useful.

Keywords: Sake yeast, Amino acids

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Analysis and Enhancement of the Ethanol Resistance of *Pichia kudriavzevii* N77-4, a Strain Newly Isolated from the Korean Traditional Fermentation Starter Nuruk, for Improved Fermentation Performance

POSTER PRESENTATION ID: 154

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Nuruk is a traditional fermentation starter used for making starch-based Korean alcoholic drinks. The yeast strain *Pichia kudriavzevii* N77-4 was newly isolated from *nuruk*. Resistance to ethanol stress is a crucial characteristic in alcoholic beverage production, but the ethanol stress-resistance mechanism of *P. kudriavzevii* remains to be clarified. In this study, we attempted to analyze the ethanol resistance mechanism of *P. kudriavzevii* N77-4, and to improve it by mutagenesis.

Gene expression was analyzed by real-time PCR under ethanol stress condition. Accumulation of intracellular reactive oxygen species (ROS) was analyzed using H2DCFDA as a ROS probe under ethanol stress condition. Ethyl methanesulfonate (EMS) mutagenesis was performed according to a standard protocol.

P. kudriavzevii N77-4 was found to show lower ethanol resistance than the *S. cerevisiae* lab strain BY4743. After 4 h cultivation under a 5% ethanol condition, the intracellular ROS levels of both N77-4 and BY4743 increased more than 8-fold compared with that under a 0% ethanol condition. Moreover, we found that the level of ROS in N77-4 increased by 40% compared with that in BY4743 under the 5% ethanol condition, indicating that the ROS accumulation level was linked to the ethanol sensitivity level in *P. kudriavzevii* N77-4 and *S. cerevisiae* BY4743. Exposure of N77-4 to ethanol stress induced a more than 3-fold increase in the expression of *S. cerevisiae SOD2*-like gene (*PkSOD2*), which may encode a superoxide dismutase, suggesting that the strain was able to generate *PkSOD2* to decrease ROS. EMS mutagenesis was performed to improve the ethanol resistance of N77-4. An isolated mutant, HER8, exhibited higher ethanol resistance, a 50% decrease in ROS accumulation, and enhanced expressions of *S. cerevisiae GPX2*-like gene, which may encode a glutathione peroxidase, and *S. cerevisiae SOD1*-like gene (*PkSOD1*). Moreover, HER8 showed 10% greater ethanol production under a high concentration of glucose condition, suggesting that up-regulation of these antioxidant genes is important for improving ethanol resistance in *P. kudriavzevii*, and use of the HER8 strain will contribute to improving the brewing fermentation.

Keywords: *Pichia kudriavzevii*, Ethanol resistance

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Microbial Diversity of Comiteco, an Agave Sap Spirit from Chiapas, México

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Comiteco is a traditional Mexican spirit produced in the Meseta Comiteca in the State of Chiapas. It is obtained from the fermentation and distillation of the mixture of agave sap (from *Agave americana* or *A. salmiana*) with piloncillo and water. The mixture is usually spontaneously fermented and then distilled in copper or stainless stills. This spirit has cultural, social and economic importance reaching a great regional relevance. The production of comiteco is mainly artisanal, although recently it has been tried to scale it to a semi-industrial level. For this, it is necessary to know the microbiota involved in the fermentation process. The only work on this topic reports the isolation of five yeasts, whose identity is still unknown. The aim of this study was to quantify and identify the microbiota present during the fermentation of this spirit, contributing to its microbial knowledge. Also, several physicochemical parameters were measured.

Samples (18) were collected from a producer in Comitán de Domínguez, Chiapas, Mexico. For microbial quantification and identification, culture dependent methods were used. After the microbial counts, 861 isolated were obtained (687 yeasts and 174 bacteria), which were identified by polyphasic taxonomy. Also, the pH, temperature, total acidity and °Brix were measured. The microbial counts of yeasts varied from 10^3 to 10^1 CFU x mL^{-1} and those of bacteria from 10^3 to 10^2 CFU x mL^{-1}. Throughout the fermentation the pH varied from 3.7 to 3.4; temperature form 27 to 20 °C; total acidity from 0.02 to 0.12 and °Brix from 27.8 to 30.1. The species present at the beginning of the fermentation were: Yeasts, *Candida ethanolica, C. rugosa, Clavispora lusitaniae, Dekkera anomala, Galactomyces candidus, G. silvicola, Hanseniaspora osmophila, H. uvarum, Kazachstania hellenica, Kluyveromyces marxianus, Meyerozyma caribbica, Pichia membranifaciens, Rhodotorula mucilaginosa, Torulaspora delbrueckii, Wickerhamiella pararugosa, Wickerhamomyces anomalus, Zygosaccharomyces baillii*; Bacteria, *Bacillus pumilus, Acetobacter malorum, A. pasteurianus, Gluconobacter cerinus, G. oxydans, Lactobacillus hilgardii, L. buchnerii, Leuconostoc citreum, L. mesenteroides, Micrococcus luteus, Staphylococcus epidermidis, S. haemolyticus.*

As the fermentation progressed, the microbial diversity decreased. At the end of the process the dominant species were *K. marxianus, A. malorum, L. citreum* and *L. mesenteroides*. The results obtained in this study will be useful to design an inoculum to obtain a homogeneous quality distillate at a semi-industrial level that satisfies the taste of the habitual consumers.

Keywords: Comiteco, *Agave* sap, Piloncillo, Spirit, Microbial diversity

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Regulatory Mechanism of Ethanol Fermentation Mediated by the Yeast Ubiquitin Ligase Rsp5

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Ethanol fermentation in the budding yeast *Saccharomyces cerevisiae* has attracted great attention because of its high ethanol productivity. Recently, we found that the substitution of Ala401 into Glu in the yeast Nedd4-family E3-ubiquitin ligase Rsp5 (*rsp5*^{A401E}) leads to decreasing total carbon dioxide emission during ethanol fermentation, suggesting that the *rsp5*^{A401E} mutant is less efficient in ethanol production. Despite the pertinent contributions of Rsp5 in various cellular functions, the role and importance of Rsp5 in the glucose metabolism and the ethanol fermentation have been poorly understood. Therefore, this study aims to investigate the potential roles of Rsp5 in glucose metabolism and ethanol fermentation processes in *S. cerevisiae* cells.

To get more insights of regulatory mechanism of ethanol fermentation mediated by Rsp5, the metabolomic profiling of wild-type and *rsp5*^{A401E} mutant cells at 24-hour fermentation has been conducted. These profiles showed that the *rsp5*^{A401E} mutant cells accumulated high levels of intracellular pyruvate, tricarboxylic acid (TCA) intermediates, and amino acids, also produced less ethanol. The changes of these metabolites suggest that Rsp5 is involved in coordination of amino acids and carbon metabolisms in yeast cells. Here, we showed that high intracellular amino acids in the *rsp5*^{A401E} mutant negatively correlate with ethanol fermentation performance. This abrogated intracellular amino acid homeostasis in the *rsp5*^{A401E} mutant was potentially due to the failure of amino acid permease downregulation, leading to the excessive uptakes of amino acids from the culture medium.

We hypothesize that Rsp5 has a pivotal role on intracellular amino acids homeostasis by downregulating permeases for efficient ethanol production.

Keywords: Ethanol fermentation, Ubiquitin ligase, Rsp5, Amino acids

Identification and Sake-Brewing Characteristics of Yeast Strains Isolated from Natural Environments in Gifu, Japan

POSTER PRESENTATION ID: 163

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In this study, 25 strains of the budding yeast *Saccharomyces cerevisiae* were isolated from several samples obtained from various natural environments in Gifu Prefecture in order to develop novel types of *sake* yeast. Among the isolated strains, strains GY38, GY115, GY156, and GY172 were selected for a novel Gifu University *sake* yeast strain. By phylogenetic analysis, strain GY115 was classified into the *sake* yeast group, while strains GY38 and GY156 were classified with baker's yeast strains. Strains GY38, GY115, and GY172 had comparatively high fermentation abilities at 15ºC, though their abilities were lower than that of strain Kyokai No. 7. These strains showed individual production abilities for organic acids and aromatic compounds: the *sake* produced by strain GY115, for example, contained comparatively high levels of ethyl caproate, but the strain could not produce the 4-vinyl guaiacol (4-VG) that results in a phenolic off flavor in *sake*. On the other hand, the *sake* produced by strain GY38 had a high malic acid/succinic acid ratio (0.77) and low succinic acid concentration. Strain GY115 was able to make *sake* on a comparatively large scale fermentation although the *sake* contained high concentrations of organic acids, including succinic acid. From these results, it is expected that GY115 has a unique ability to produce specific desirable flavors for *sake* production.

Keywords: *Sake* yeast strain, *Saccharomyces cerevisiae*, Isolation, 4-VG

Can Community-Based Signaling Behavior in *Saccharomyces cerevisiae* Be Called Quorum Sensing? a Critical Review of The Literature

POSTER PRESENTATION ID: 169

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Quorum sensing is a well-described mechanism of intercellular signalling among bacteria that involves cell-density dependent chemical signal molecules. The concentration of these quorum sensing molecules increases proportional to cell density until a threshold value is exceeded which triggers a community-wide response.

In this review we propose that intercellular signalling mechanisms can be associated with a corresponding ecological interaction type based on similarities between how the interaction affects the signal receiver and producer. Thus, we do not confine quorum sensing, a specific form of intercellular signalling, to only cooperative behaviours. Instead we define it as cell-density dependent responses which occur at a critical concentration of signal molecules and through a specific signalling pathway. For fungal species, the medically important yeast *Candida albicans* has a well-described quorum sensing system while this system is not well described in *Saccharomyces cerevisiae*, which is involved in food and beverage fermentations. The more precise definition for quorum sensing proposed in this review is used to consider the studies suggesting that *S. cerevisiae* may undergo intercellular signalling through quorum sensing.

We conclude that there is a lack of evidence to support a specific signalling mechanism and a critical signal concentration of these behaviours in *S. cerevisiae* and thus these features require further investigation.

Keywords: Quorum Sensing, Intercellular Signalling, *Saccharomyces cerevisiae*, Yeast, Microbial Interactions

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An Application of Yeasts *Saccharomyces cerevisiae* and *Kluyveromyces marxianus* for Purification of Galactooligosacharides Produced from Milk Permeate

POSTER PRESENTATION ID: 172

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Galactooligosaccharides (GOS) are prebiotics with growing application as functional food ingredients. GOS are mostly produced by beta-galactosidases from pure lactose or whey or milk permeate obtained after separation of proteins. GOS obtained in the lactose bioconversion by beta-galactosidases contains up to 60% of GOS and by-products – glucose, galactose and residual lactose. GOS concentration in product can be increased by removal of other sugars using selective fermentations by microorganisms. In the present study the application of yeasts *S. cerevisiae* and *K. marxianus* for selective removal of sugars from GOS obtained from milk permeate was investigated.

S. cerevisiae and *K. marxianus* was added to the GOS mixture obtained from the enzymatic conversion of lactose containing concentrated milk permeate by *Bacillus circulans* β-galactosidase. The fermentation by yeasts was done in the presence of still active β-galactosidase. The GOS mixture was diluted prior addition of yeasts to decrease osmolality of reaction mixtures because GOS enzymatic biosynthesis are done at high lactose concentrations (50% of DW). The impact of substrate concentration, yeast inoculum amount and aeration rate on efficiency of carbohydrate removal process was studied. In the case of *K. marxianus* higher aeration rate was necessary. The dynamic of different carbohydrate utilization was studied. All free glucose was consumed in 25-30 hours by both yeasts. Decrease in lactose concentration in the case of *S. cerevisiae* was due to hydrolytic action of *B. circulans* β-galactosidase with consequent utilization of glucose and galactose by yeasts. Co-fermentation by *S. cerevisiae* with β-galactosidase increased GOS content in mixture up to 80% of DW. Higher concentration of GOS was obtained by 72-hour co-fermentation of yeasts *K. marxianus* with *B. circulans* beta-galactosidase as compared with *S. cerevisiae*. In this case GOS content increases up to 95% (from DW) but the molecular weight profile of GOS was significantly altered because of ability of *K. marxianus* to utilize not only lactose but also low molecular weight GOS. The GOS with DP2 decreased from 36% to 9,7% of total GOS. The proportions of GOS fractions with DP4 and higher increased comparing with initial mixture.

It can be concluded that *K. marxianus* can be used not only for purification of GOS but also for modifying of GOS mixture composition to increase GOS content with higher molecular weight.

Keywords: *Saccharomyces cerevisiae*, *Kluyveromyces marxianus*, Galactooligosaccharides, beta-galactosidase, Lactose

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Molecular-genetic Peculiarities of Pectinase Genes in *Saccharomyces* Yeasts

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Pectinase (endo-polygalacturonase) is one of the main enzymes involved in hydrolysis of plant pectin. This enzyme plays an important role in clarification of fruit juices, must and wine. In wine making, there are two essential *Saccharomyces* yeasts: *S. cerevisiae* and *S. uvarum*. The latter yeast is usually associated with the production of white, sweet and sparkling wines, and cider (Naumov et al. 2011).

Using sequence analysis, molecular karyotyping and Southern hybridization, we studied the polygalacturonase-encoding *PGU* genes in 84 *Saccharomyces* (*S. cerevisiae* and *S. uvarum*) strains isolated from various fermentation processes and natural sources in different world regions: Europe, Australia, North and South America. Screening for polygalacturonase activity was conducted by the plate assay (Louw et al. 2010).

Unlike *S. cerevisiae* having single *PGU* gene, all 74 *S. uvarum* strains studied revealed three divergent *PGU1b*, *PGU2b* and *PGU3b* genes, the nucleotide sequences of which are similar by 86.1–95.7%. At the same time, the similarity of *PGU* genes of *S. cerevisiae* and *S. uvarum* did not exceed 80%. Using molecular karyotyping and Southern hybridization, the *PGU1b*, *PGU2b*, and *PGU3b* genes were mapped on the chromosomes X, I and XIV, respectively. All *S. uvarum* strains showed pectolytic activity, while half of the *S. cerevisiae* strains were unable to degrade pectin.

The molecular genetic analysis of pectinase genes showed that, regardless of the source and location of the isolation, the strains of *S. uvarum* have the genotype *PGU1b PGU2b PGU3b.* Apparently, the high pectinolytic activity of this yeast is associated with the presence of several *PGU* genes having cumulative effect. In industrial *S. cerevisiae* strains, the accumulation of polymeric genes of sugar fermentation is known to lead to intensification of the fermentation process.

Keywords: Endo-polygalacturonase, *PGU* gene, pectin, Wine yeasts, *Saccharomyces uvarum*

Acknowledgements: The study was supported by the Russian Foundation for Basic Research (project no. 17-04-00309).

Peptides Assimilation by Yeast and Their Role on Esters Synthesis

POSTER PRESENTATION ID: 186

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Nitrogen deficiency is one of the most common fermentation related issues encountered by fermentation industry. Insufficient concentrations of yeast assimilable nitrogen (YAN) have negative impact on yeast fermentation kinetics as well as on the synthesis of several aromatic esters which precursors are some amino acids. Besides conventional inorganic ammonium sources such as mono- or diammonium phosphate (MAP and DAP, respectively) yeast autolysates are gaining popularity as complex nutrient sources. Peculiarity of the latter is that they contain a wide variety of peptides that may well serve as the nitrogen source to fermenting yeast but which are not considered as YAN, mostly due to technical challenges related to assessment of their assimilation by yeast. In the present work we studied peptides assimilation ability by wine yeast *S. cerevisiae* and the effect of peptides on fermentation kinetics and esters synthesis in comparison with MAP.

BSA (Bovine Serum Albumin) tryptic hydrolysate with the defined composition of peptides was used as the source of peptides. Synthetic grape must (SGM) with different initial concentrations of YAN was supplemented either with peptides from the tryptic hydrolysate or ammonia from MAP. Fermentation kinetics was determined by gravimetric method, the assimilation of peptides was determined based on their relative concentrations by LC-MS, and the relative concentrations of ethyl and acetate esters were determined by SPME-GC/MS.

The addition of peptides into YAN deficient SGM resulted in higher fermentation rate and ethanol production compared to ammonia addition, suggesting beneficial effect of peptides on fermentation kinetics. Under these conditions most of the di- and tripeptides were consumed simultaneously with NH_4^+ by the end of the active fermentation phase (60h) whereas larger peptides (tetra- and pentapeptides) were consumed more slowly. By contrast, slow consumption of di- and tripeptides took place when peptides were added into the nitrogen abundant SGM, suggesting that the concentration of free NH_4^+ seems to be critical to determine yeast's capability to take up peptides. The initial YAN concentration had a significant effect on acetate and ethyl esters synthesis when different nitrogen treatment was applied, in particular when peptides were added as additional nitrogen source into YAN deficient SGM. By contrast, when peptides were added into SGM with high initial YAN concentration, their positive effect on esters synthesis could not be demonstrated.

Keywords: Peptides, Aroma, Yeast, Wine

Yeast Diversity in Fermentations of Different Fine Cocoa Varieties Achieved in Two Locations in Chiapas, Mexico.

POSTER PRESENTATION ID: 189

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Different varieties of fine cocoa are still present in Mexico, mainly in the State of Chiapas. These varieties are very sought after for the complex aromatic characteristics they present. Before being the raw material for the elaboration of chocolate, cocoa beans go through three important processes: fermentation drying and roasting. The fermentation of cocoa is very complex and involves the intervention of a large number of yeasts and bacteria, which allow the generation of volatile compounds and key precursors of the final aroma of the raw material. In this work 5 different varieties of cocoa were fermented in two locations in the state of Chiapas and the different populations of yeasts were monitored throughout the fermentation to determine the impact of the fermentation site on the diversity of the yeasts present.

A series of spontaneous fermentations of the 5 varieties of cocoa were carried out in parallel in two different locations. The cocoa pods were transported to the fermentation site and broken on site. The fermentations were carried out in clean wooden boxes, a daily sampling was carried out for 5 or 6 days. The yeasts were isolated in WL medium and identified by MALDI-TOF MS and ITS sequencing.

The varieties of cocoa used are 2 Trinitario types (“Arcoiris” and “Regalo de Dios”) and 3 Criollo types (“Carmelo”, “Rojo Samuel”, “Lagarto”). Each variety is characterized by the weight and appearance of its pods (size, shape and color) and by the number of beans present per pod. In the fermentation of these different kinds of beans, the yeasts were detected throughout all the fermentations in comparable populations. A total of 23 species belonging to 11 different genera were isolated. The two species that predominate at the beginning of almost all fermentations are *Hanseniaspora opuntiae* and *Pichia kudriavzevii*. These two species are very common in cocoa beans fermentations. A difference between the two locations where the fermentation took place is clearly detected. The fermentations are much more complex in location 1 (Cacahuatán) than in location 2 (Tapachula). For example, *Pichia manshurica* is present only in the fermentations at locality 1. In total, 6 yeast species were detected only in location 1.

In conclusion, the place where the fermentation is carried out determines the number and species of yeast which will achieve the fermentation. Now it is important to determine if these variations in yeast populations have an impact on the aromatic characteristics of the cocoa liquor.

Keywords: Cocoa varieties, Yeast, Fermentation

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Production of Volatile Aromatic Compounds for Bakery with Some Yeast Species

POSTER PRESENTATION ID: 203

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The bread aroma is major sensory property for consumers. However, to meet the high consumer's demand, the aromatic wellness of bread has been lost due to the fast-industrial production where insufficient fermentation is occurred. In this respect, use of aroma mix pre-prepared by fermentation while producing the bread dough would be one supplementary approach. The aim of this study was to determine and compare the production levels of volatile aromatic compounds (VACs) that are important at bread flavour by using food-sourced two *Saccharomyces cerevisiae* (PFC107 and PFC121) and one *Kluyveromyces marxinaus* (PFC120) under batch fermentation system on cereal-slurries prepared with 10% wheat, oat and rye flour.

The slurries were sterilized after treated with amylolytic and proteolytic enzymes. The yeasts inoculated 5 logCFU/ml to the relevant slurries were incubated at 30°C and 150 rpm agitation for 48 h. Subsequently the yeast growth was followed and VACs were determined at GC/MS equipped with SPME.

The yeast species were evaluated for the production of 13 VACs important bread flavour. Yeasts produced more VACs at oat and rye slurry than wheat slurry used. All yeasts produced different amounts of 2-methyl-1-propanol, 3-methyl-1-butanol, ethyl acetate, ethanol, benzeneethanol. Acetaldehyde, 2-phenylethyl ester, 6-methyl-5-hepten-2-one and octan-2-one were only determined at oat and rye flour slurries. 1-propanol was only produced at rye flour slurry. Additionally, among the yeasts *S. cerevisiae* PFC121 produced the highest amounts of VACs at all cereal-slurries used.

In conclusion, *S. cerevisiae* PFC121 was capable VACs producer related with the bread flavour and can be used for preparing directly applicable aroma mix with using cereal-flour slurries to enhance and strengthen the aromatic structure of the bread.

Keywords: Volatile aromatic compounds, Bread, Yeast, *Saccharomyces cerevisiae*

Acknowledgements: This study was supported by University of Pamukkale, Scientific Research Projects Coordinatorship with grant number 2019FEBE005.

Technological Properties of Some Baker's Yeast Isolated from Turkish Sourdough

POSTER PRESENTATION ID: 205

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In this study, some of the important technological properties of *Saccharomyces cerevisiae* yeasts isolated from 7 different sourdough samples obtained from different regions of Turkey and 5 samples of baker's yeast obtained from the Culture Collection of the Food Engineering Department of Ankara University were evaluated in an attempt to select yeast strains potentially applicable in the baking industry.

The technological properties evaluated were the following: yeast growth, leavening ability, pH and total titratable acidity. Yeast growth was monitored by either measuring the optical density of the culture at 600 nm (OD600) or by microscopic counting on Thoma slides after 4th, 8th and 24th h of incubation. For the determination of leavening ability, the dough samples prepared with yeast strains were incubated at 35 °C for 4 h, and the leavening ability of the yeast strains was determined by recording the hourly changes in dough volume. The pH and total titratable acidity of the dough samples were determined after incubation at 35 °C for 4 h.

It was revealed that the yeast isolates completed a significant part of their growth within the first 8 hours of incubation, and the yeast counts were in the range of 5.5-9.8 x 10^8 cells/mL after 24 hours of incubation. With respect to the leavening ability, it was determined that the leavening was almost completed within the first two hours, with values ranging from 120.0 to 152.7%. It was found that the amount of change in dough swelling ranged between 136.5 and 166.2% after 4 hours. The E12 and E7a isolates, which were found to have the highest leavening ability, showed good potential for use as starter culture in bread production. The results indicated that the pH values and the total titratable acidity ranged between (5.2-5.9) and (3.5-5.1 g/L), respectively.

As a result, from a technological point of view, it is important to note that the investigated yeast samples were shown to have potential to be used in bread production. The results obtained from this study gave insight for further studies on the development of starter cultures for baking industry.

Keywords: Sourdough, Baker's yeast, Technological properties

Molecular Studies on Yeast Diversity in Bulgarian Sourdoughs

POSTER PRESENTATION ID: 219

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Yeasts and lactic acid bacteria are the most commonly found in artisanal sourdoughs around the world. They form stable mixed populations, with composition defined by a number of variables, such as the raw materials used, geographical factors, preparation methods, and production hygiene. In Bulgaria, sourdough tradition was almost lost during the second half of the 20th century due to the very limited product diversity in bread production. However, in the past 15 years consumer's interest to sourdough baked goods is on the rise because of their distinct flavor, texture and healthy attributes. This trend currently employs the efforts of researchers and industry to implement tradition and modern scientific approaches into developing new sourdough products. There is hardly any scientific data on Bulgarian traditional sourdoughs and, therefore, the aim of the current study was to investigate the diversity of yeasts in sourdoughs from different regions of the country.

Fifteen sourdoughs (SDs) prepared by traditional methods were collected from different locations across the country. The samples were analysed for dry matter content, pH and titratable acidity (TA). Total yeast counts in the samples were also assessed. Pure yeast strains were isolated and subjected to phenotypic analysis. Molecular identification was further performed by PCR amplification and sequencing of the ITS1-5.8S-ITS2 region. Phylogenetic analysis was also performed on all identified yeast strains using the CLUSTALW platform.

Physicochemical analyses of the SDs showed dry matter content from 25.17 to 58.16%, pH values within 3.57 and 5.40, and TA from 4.3 to 12.4. Results proved that acid production was more abundant in sourdoughs with higher moisture content. Total yeast counts were highly variable, with values between $1x10^4$ and $8x10^7$ cfu/g. Again, low dry matter content was associated with high yeast counts. Molecular identification of the yeasts showed that 68.38 % out of the total isolates belonged to *Saccharomyces cerevisiae*. The other species found were *Kazachstania barnettii, K. unispora, K. humilis, K. servazzii, Pichia fermentans, P. membranifaciens, Yarrowia lipolytica* and *Candida glabrata.*

These results are the basis for further studies of the microflora of traditional Bulgarian sourdoughs. The effects of different factors on the microbial communities and their technological performance also need to be revealed. This knowledge will give the foundation for further development of defined starter cultures and for establishment of industrial technologies to meet the growing consumer demand of sourdough products with improved and stable characteristics.

Keywords: Yeast, Sourdough

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Yeast Cell Death Caused by Nutrient Desequilibrium During Alcoholic Fermentation is Impacted by Nitrogen Sources

POSTER PRESENTATION ID: 220

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Nutrients availability is a key factor for controlling wine alcoholic fermentation. Among them, nitrogen has been identified as an essential parameter, controlling both the fermentation rate and the duration of the fermentation. However, nitrogen is not sufficient to ensure a correct fermentation and other nutrients such as vitamins and lipids, present in lower quantities, are required. Furthermore, we showed in a previous study that an excess of nitrogen combined with a depletion in certain micronutrients can lead to cell death and sluggish or stuck fermentation. In this study, we provide evidence of the mechanism controlling cell death and we show that all the nitrogen sources are not equivalent in the initiation of this phenomenon.

Fermentations limited in oleic acid, pantothenic acid and nicotinic acid showed yeast cell death linked to a high nitrogen content. In each case, lowering the nitrogen level restored yeast viability. We evidenced that yeast cell lack of a correct stress response to those micronutrient starvations in presence of high levels of nitrogen. A transcriptional analysis showed a correct stress response suggesting that the lack of resistance originates from a post-transcriptional control mechanism. We then provide evidence that the nitrogen Tor/Sch9 signaling pathway is involved in triggering cell death.

Yeast cell viability was then monitored and compared during fermentation starting at different nitrogen levels, with the addition of different nitrogen sources (19 amino acids and NH4+) and two different timing of NH4+ addition. We observed that cell death was triggered with different intensities.

Yeast cell death associated to disequilibrium between micronutrients and nitrogen has been evidenced and its implication on fermentations highlighted. We showed a strong impact of both the nature of the nitrogen source and time of addition on yeast cell death and fermentation outcome.

Keywords: Wine yeast, Nitrogen, Cell death

The Power of Sour – Brewing Potential of *Lachancea thermotolerans*

POSTER PRESENTATION ID: 229

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Sour beers are increasingly popular among craft breweries and beer enthusiasts alike. Typically, they are produced by mixed cultures of both yeasts and lactic acid bacteria, with the latter driving the acidification. The yeast *Lachancea thermotolerans* is characterised by abundant lactic acid production concomitant to alcoholic fermentation. This work therefore focused on selection and characterisation of *L. thermotolerans* strains to be used as an alternative to bacteria in sour beer production. For this purpose, 20 *L. thermotolerans* strains were grown in malt extract (SG 1.047; pH 5.27) and compared for their fermentation kinetics parameters, attenuation and acidification effect. The pH of the obtained beers varied between 3.38 and 4.08. Six strains were chosen for further trials in mixed culture fermentations with *Saccharomyces cerevisiae* (US-05). The acidification effect and fermentation dynamics depended on the *L. thermotolerans* yeast strain, co-inoculation rate (1:10 and 1:100) and the delay of *S. cerevisiae* inoculation (2 days and 9 days). Finally, pilot-scale beers (19 L) were produced using two *L. thermotolerans* strains with sequentially inoculated *S. cerevisiae* (after 2 and 7 days), alongside an *S. cerevisiae* control. The beers were analysed for their basic chemical composition, volatile profile (HS-SPME-GC-MS) and sensory properties. The mixed-culture *L. thermotolerans* beers (pH 3.5) significantly differed from the *S. cerevisiae* monoculture (pH 4.1) in a range of chemical and sensory parameters, without any faults. No obvious benefits were seen from postponing the *S. cerevisiae* inoculation from 2 to 7 days. Altogether, results show the feasibility of producing high-quality sour beers using *L. thermotolerans* as an alternative to lactic acid bacteria.

Keywords: Non-*Saccharomyces* yeasts, *Lachancea thermotolerans*, Brewing, Sour beer

Pichia kluyveri: A Yeast with an Interesting Potential to Produce Volatile Compounds, in Particular Esters

POSTER PRESENTATION ID: 230

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In recent years the importance of non-*Saccharomyces* yeasts has been recognized in production processes of alcoholic beverages, mainly in wine, due to their ability to produce and release metabolites of interest, in particular volatile compounds as esters. *Pichia klyuyveri* is one of these strains capable of producing interesting amounts of esters. In this work, 21 different strains of *P. kluyveri* were studied coming from different fermentations of Mexican artisanal products. In particular, different growth and fermentation conditions were tested to determine which strains were able to generate high concentrations of esters.

Twenty-one different strains of *Pichia kluyveri* from the yeast collection of the Centro de Investigation y Asistencia en Tecnología y Diseño del Edo de Jalisco A.C. (CIATEJ) were used in this study. These yeasts were previously isolated from different fermentation processes such as mezcal (agave fermentation), raicilla (agave fermentation), tejuino (corn fermentation) and cocoa. A series of experiments were conducted to characterize all strains of the point of view of the production of ethanol and volatile compounds in different media (chemically defined medium and a medium based on agave juice) and in different conditions.

The first important result is the big differences in the behaviour of the different strains. Even strains of the same origin exhibit different behaviours. In terms of media, agave juice favours the production of esters compared to the chemically defined medium. The strains from mezcal fermentation produce more esters (in number of compounds and in quantity) than the strains of cocoa fermentations. In the fermentations with agave juice, the KB1b, ME4a and MG1 strains stand out. The KB1b strain produced 6 of the 7 esters analysed by gas chromatography, from the cocoa group it was the one that showed better behaviour in agave juice. The ME4a strain produced 5 of the 7 esters analysed which was a similar behaviour for the other agave strains, however, it is highlighted in the concentrations of the esters formed, mainly from ethyl acetate, producing a concentration of 335.7 mg/ L. Finally, the MG1 strain, despite having generated fewer compounds than the other strains of agave, was the one that produced the highest concentration of ethyl acetate.

This work demonstrates that *Pichia kluyveri* is an interesting yeast species to produce esters, however the selection of the strains as well as the cultivation conditions are fundamental for obtaining quantities of attractive quantities of esters.

Keywords: *Pichia kluyveri*, Fermentation, Ester production

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Influence of Sequential Inoculum of *Starmerella bacillaris* and *Saccharomyces cerevisiae* on Sangiovese Wine Quality

POSTER PRESENTATION ID: 233

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The use of selected non-*Saccharomyces* and *Saccharomyces cerevisiae* strains as mixed starter cultures in winemaking management has increased because they may have positive influences on some sensorial characteristics and the complexity of wines. In particular, selection of *Starmerella bacillaris* strains has been suggested in order to promote fructose degradation due to their fructophilic character and to produce wines containing lower ethanol content and higher glycerol concentrations. However, studies about effects on phenolic compounds in red wines are currently lacking. Therefore, the aim of this work was to evaluate the influence of sequential inoculum of *Starm. bacillaris* and *S. cerevisiae* on phenolic content of mono-varietal Sangiovese wines.

Experimental fermentations were carried out at 28°C in triplicate by using a single lot of Sangiovese grapes. In the sequential fermentation (SF), the must was inoculated with *Starm. bacillaris* followed by *S. cerevisiae* after 6 days. Pure fermentations (PF) inoculated with *S. cerevisiae* were performed as control. All the fermentations were monitored by microbiological and chemical analyses. The predominance of inoculated yeast strains was assessed by molecular techniques. Total phenol index (TPI) and colour intensity were determined by spectrophotometer assays. The individual anthocyanins were separated, identified and quantified by HPLC according to the Organisation Internationale de la Vigne et du Vin methods. The determination of flavonols and flavan-3-ols was performed by HPLC analysis.

Molecular analysis confirmed the implantation of the inoculated yeast strains. *S. cerevisiae* strain attained higher cell densities in PF. The experimental wines obtained by SF showed significant lower ethanol content and higher glycerol concentrations, whereas no significant difference was detected in colour intensity. The TPI reached significantly lower values in SF than in PF. Furthermore, the wines produced by SF contained higher concentrations of vitisin A (formed through condensation of pyruvate produced by *Starm. bacillaris* and malvidin-3-O-glucoside) which has a greater colour stability than the anthocyanins monomer. Finally, also a lower content of both free anthocyanins and flavan-3-ols, key compounds for wine quality possessing also health-enhancing properties, was found in wines obtained by SF. On the contrary, no significant difference was detected on flavonols concentration between SF and PF.

This study highlighted that the use of sequential inoculum of *Starm. bacillaris* and *S. cerevisiae* can contribute to increasing the colour stability of red wines, even if at the expense of compounds with health properties.

Keywords: Mixed culture of yeasts, Sangiovese wine

Freeze-Dried Raw Materials for Low Temperature Wine Making Using Pine Sawdust (*Pinus halepensis*) Entrapped Cells as a Promoter

POSTER PRESENTATION ID: 240

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In this study, a new methodology is proposed for low temperature sweet wine- making. Pine sawdust was delignified and the received porous delignified cellulosic material (or tubular cellulose; abbrev TC) was used as carrier for immobilization of the psychrotolerant and alcohol-resistant yeast strain *Saccharomyces cerevisiae* AXAZ-1.

The immobilization of yeast cells on TC was examined by SEM. Various concentrations of freeze-dried immobilized biocatalyst and high-density grape must were mixed for fermentation at various temperatures (1-10^{o}C). The fermentation efficiency of the dried mixtures was evaluated during various time intervals of storage after addition of suitable amounts of drinkable water. The amount of added water was also examined in order to produce wines with high alcohol content and low residual sugar concentration. The effect of temperature on the fermentation kinetics was evaluated. The volatile fraction of sweet wines was analyzed using headspace solid-phase microextraction (HS-SPME) followed by gas chromatography mass spectrometry (GC/MS). Finally, sensory evaluation was performed.

The immobilized biocatalyst was found efficient for high gravity grape must fermentations at low temperature and high final alcohol concentration. The effect of temperature (1-10^{o}C) on the fermentation kinetics revealed the fermentation efficacy of the immobilized biocatalyst at low temperature providing lower production costs. GC-MS analysis and sensory evaluation revealed the promotional effect of TC on formation of volatile by-products in comparison with free cells.

Winemaking comprises a diverse set of factors that play a crucial role during the transformation of grapes to wine. Increasing consumer demand for good quality wine of low costs has led to the manufacture of a novel marketable dried mixture for high-quality wine making at low temperature. This novel dried mixture consisted of tubular cellulose with entrapped psychrotolerant yeast cells as a promoter. The produced high-quality wines were free of preservatives and they could alternatively be prepared by the addition of fresh juices depending on consumers demand.

Keywords: Sweet wine-making, Psychrotolerant yeast, Tubular cellulose, Freeze-drying, Low temperature.

Effect of Yeast Strain on Esters Production in Wine

POSTER PRESENTATION ID: 253

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Wine has very complex aromas. Large part of aroma compounds in wines is composed by the action of yeasts during the alcoholic fermentation. Depending on the yeast strain, the concentration of aroma compounds is determined and changing the sensory perception of wine. Especially, as a one of the yeast by-products, esters are important compounds due to the higher impact on wine aroma. Their production by yeast during the fermentation have significant effect on the fruity flavors in wine. Most of the ester have concentration over their threshold value. Small changes in their concentration have impressive effect on wine flavor. The level of ester production by yeast strain is a characteristic for starter selection. However, the presence of different esters can have a synergistic effect on the individual flavors. Esters are produced by mainly yeast metabolism. The ester production rate influenced by the concentration of alcohol and activated acyl-CoA molecule. Ester formation is enzyme-catalyzed reaction and it depends on the ester synthase activity which differs according to yeast strain. Acetic esters of higher alcohols is formed via the condensation of an alcohol and activated acyl-CoA molecule that is catalyzed by alcohol acetyltransferases (AATase). *Saccharomyces cerevisiae* has two AATases which are *Atf1p* and *Atf2p* encoded by *ATF1* and *ATF2* genes, respectively. And also *S. bayanus* have another AATase, the *Lg-Atflp*, encoded by *Yscatf1* gene. The *Atf1p* is the main enzyme for the production of the acetate esters like isomayl acetate, phenyl ethyl acetate and C3 to C8 acetate esters. The *ATF* overexpression research reported that acetate ester production is increased by the high level of these enzymes. Fatty acids ethyl esters as like ethyl butyrate (C4), ethyl hexanoate (C6), ethyl octanoate (C8) are synthases via esterification of ethanol with medium-chain fatty acyl-CoA (MCFA). The two main enzymes, encoded by *EHT1* and *EEB1*, have been reported as being responsible for the ethyl esters production. Overexpression of *EHT1* in commercial wine strain slightly increases ethyl esters producing more fruity. Moreover, level of MCFA directly affect the ethyl ester concentration in wines. And also non-*Saccharomyce*s wine yeast such as *Hanseniaspora guilliermondii* and *Pichia anomala* that can contribute to the ester aromas of wine. Yeast strain is one of the major factor affecting ester production. Beside ester concentration impacted and also proportion of the individual esters differ dramatically from strain to strain.

Keywords: Yeast Strain, Ester, Aroma

Yeasts as Sources of Ingredients and Additives

Efficient Production of α-Ketoglutaric Acid from Mixture of Rapeseed Oil and Glycerol Using Genetically Engineered *Yarrowia lipolytica*

POSTER PRESENTATION ID: 128

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Yarrowia lipolytica is rapidly gaining popularity as an industrial host for production of various chemicals including α-ketoglutaric acid (KGA). It is used in dietary supplements as a direct precursor for glutamine and glutamate. The thiamine-auxotrophic *Y. lipolytica* is a robust KGA producer which is mainly synthesized through the TCA cycle. In a recent report various strains of *Y. lipolytica* were employed for the production of KGA on media containing glycerol, ethanol and vegetable oils as carbon sources.

The aim of the presented work was to develop an efficient process of KGA production from a mixture of rapeseed oil and glycerol by different genetically engineered *Y. lipolytica* strains.

Y. lipolytica A-101.1.31 strain was engineered to overexpress glycerol kinase (GUT1, YALI0F00484g), methylcitrate synthase (CIT1, YALI0E00638g) and putative succinate-fumarate transporter (ACR1, YALI0E34672g). The genes were inserted separately or in combination of GUT1 and CIT1, CIT1 and ACR1 as well as GUT1, CIT1 and ACR1. The genes were controlled by strong constitutive TEF promoter and were randomly inserted into the yeast genome using retrotransposon (ZETA) sequences.

All bioreactor culturing took place in a 5-L stirred tank reactor, the working volume was maintained at 2-liters. The process of KGA production was started as a batch culture in medium consisting of rapeseed oil 20-25 g/L, nitrogen and phosphorus sources and 3 µg/L of thiamine. After 48 h of batch culture 60 g/L of glycerol was added into bioreactor. Next, after 96-110 h of batch culture, rapeseed oil in concertation of 25 g/L was added to bioreactor. The pH was maintained automatically at 3.0-3.5 by the addition of 20% $Ca(OH)_2$ solution.

It was found that new engineered strains can produce KGA with high selectivity from two renewable carbon sources - rapeseed oil and glycerol. The concentration of KGA varied from 40 to 65 g/L and depended on the strain used. In these processes pyruvate acid was produced as a by-product in the range of 4 - 35 g/L.

The results of this study represent a good starting point for the research on further optimization of culture conditions for integrated fed-batch strategy.

Keywords: Ketoacids, Glycerol, Rapeseed oil, Fed-batch culture

Acknowledgements. This study was financed under Project No. POIR.04.01.02-00-0028/18 entitled "Development of an innovative technology for the production of a dietary supplements based on alpha-ketoglutaric acid obtained on the biological way with *Yarrowia lipolytica* yeast."

Factors Affecting Pyruvic Acid Biosynthesis from Glycerol by *Yarrowia lipolytica* Yeast

POSTER PRESENTATION ID: 129

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Modern biotechnology faces the necessity of creating new technologies based on environmentally friendly and economically profitable production processes. The use of yeast *Yarrowia lipolytica*, with GRAS status given by FDA, for the production of valuable compounds such as pyruvic acid (PA) using waste substrates perfectly fits this trend. Therefore, the aim of the performed study was to examine the impact of different factors on biosynthesis of PA by *Y. lipolytica* and what follows, propose a commercially attractive process of PA production. The influence of the following factors was examined: type of glycerol used, C:N ratio, thiamine concentration, biotin addition, pH of cultivation medium, stirrer speed, as well as the possibility of process intensification through the use of fed-batch culture.

The wild type strains of *Y. lipolytica*: A-10, SKO6 and an acetate negative mutant Wratislavia 1.31 were used in this study. The production cultures were carried out at 30°C in a 5-L stirred-tank reactor with a working volume of 2 L. The pH was maintained automatically by addition of NaOH solution.

Performed studies have shown that pure glycerol was a very good substrate for the biosynthesis of PA by *Y. lipolytica*; the use of waste glycerol requires additional testing, due to the variable composition of this substrate; there were no significant changes in PA production parameters while changing C:N ratio (from 17.9, 26.8, to 32.2); addition of biotin to the culture medium inhibited the biosynthesis of PA; for efficient production of PA the thiamine concentration was depended on the type of glycerol (pure or waste); pH range from 3.5 to 4.5 was optimal for the production of PA from pure and waste glycerol; stirrer speed of 800 rpm and aeration rate of 800 vvm were indicated as optimal.

In case of the strain SKO 6 optimization of culture conditions in batch culture allowed for an increase in PA concentration by 37%, when compared to the culture without optimization. In the fed-batch culture 125.8 g/L of PA was obtained with the yield of 0.68 g/g and volumetric production rate of 0.58 g/Lh, which is the highest concentration reported so far for PA production by *Y. lipolytica* yeast.

Keywords: *Yarrowia lipolytica*, Pyruvic acid, Crude Glycerol

Acknowledgements. This study was financed under Project No. POIR.04.01.02-00-0028/18 entitled “Development of an innovative technology for the production of a dietary supplements based on alpha-ketoglutaric acid obtained on the biological way with *Yarrowia lipolytica* yeast.”

Optimization of Kynurenic Acid Biosynthesis in *Yarrowia lipolytica*

POSTER PRESENTATION ID: 144

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Kynurenic acid (KYNA) is the product of tryptophan metabolism and it is synthesized by kynurenine aminotransferases via transamination of kynurenine. KYNA exhibits pro-health properties i.e. antioxidant, anti-inflammatory, anti-atherosclerotic action therefore its supplementation has been proposed. The aim of this work was to optimize synthesis of KYNA in yeast *Yarrowia lipolytica*. Our former studies showed that this metabolite can be synthesized by *Y. lipolytica* strain S12, when fructose was used as a source of carbon. Thus the impact of different honeys, which are rich source of both fructose and KYNA were tested in present study.

The 168-h bioreactor cultures in optimized conditions with supplementation of lime-tree honey or alternatively buckwheat honey, acacia honey, rape honey, polyfloral honey, honeydew honey and chestnut honey were performed. The quantitative analyses of KYNA via HPLC method were carried out in culture broth and yeast biomass.

The concentration of KYNA in culture broth and as well in biomass yeast depended on the kind of honey used. The obtained results showed that the most efficient substrate for KYNA production is chestnut hone. The amount of determined KYNA level was up to 199 mg/L in culture broth and 959 mg/kg in biomass.

The results of this study represent a good starting point for the research on further optimization of culture conditions for integrated fed-batch strategy. The results are very promising as they may lead to the development of a low-cost process for the production of *Y. lipolytica* biomass rich in KYNA for humans. A new Novel Food "*Yarrowia lipolytica* heat-killed biomass yeast**"** will soon be authorized in the EU law and market.

Keywords: *Yarrowia lipolytica*, Kynurenic Acid, Biosynthesis, Honey

Valorization of By-Products from the Vegetable Oil Industry into High Value Products

POSTER PRESENTATION ID: 146

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Oil extraction from different oil plants, generates several by-products that can be used to feed animals, particularly the cakes and pomaces obtained from the extraction process. These plant residues are by no means waste – quite the contrary, they are a very valuable and 100% natural feed for animals because could be highly valuable sources of protein and energy. Their applicability as feed components is, however, limited due to the presence of antinutritional factors and crude fiber, the amino acid composition of protein or the fatty acid composition of oil. One of the means of their management is offered by their biotechnological conversion into an added value product with the use of microorganisms. The presented study was aimed to propose an innovative technology for the production of a new feed product enriched by *Yarrowia lipolytica* yeast biomass using by-products derived from the vegetable oil industry.

The investigations were conducted on 4 substrates: rapeseed cakes (RSC), pumpkin-seed cakes (PSC), hemp-seed cakes (HSC) and wheatgerm cakes (WGC) obtained from the oil plant industry. The wild type strain of *Y. lipolytica* S6 was used in this study. The production culture was carried out for about 48h in a 3.5 L stirred-tank reactor (AK-3) with a working volume of 1.1L at 30°C, aeration rate fixed at 1 v/v/min, and stirrer speed 600 rpm. The pH 3.75-4.0 was maintained automatically by the addition of 20% (w/v) of NaOH solution. Submerged cultures were run in the production medium containing 100 g/L of each substrate. The chemical composition and nutritional value of cakes with and without yeast biomass were characterized.

The strain S6 was able *to* growth in media containing all tested substrates. Crude protein content in dry matter of cakes with yeast was, from 30.84% to 72.56% and was higher than the amount of these components in cakes without yeast (28.37% to 58.36%). Contents of lipids in our new feed products from different substrates vary and depend on substrate type. The highest concentration of lipids, 10.83% dry biomass, was determined in RSC without yeast.

The results are very promising as they may lead to the development of a low-cost process for the enriched by-products from the vegetable oil industry by yeast biomass. The mastery of the technological process will enable us the production of new high protein, macro- and micronutrient feed products intended for the feeding of farmed as well as domestic animals.

Keywords: Oilseed Cakes, *Yarrowia lipolytica*, Biomass, Nutritional Value

Engineering Yeast to Produce Rosmarinic Acid

POSTER PRESENTATION ID: 155

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Rosmarinic acid (RA) is a hydroxycinnamic acid ester commonly found in the *Boraginaceae* and *Lamiaceae* plant families. It exhibits various biological activities, including antioxidant, anti-inflammatory, antibacterial, anti-allergic, and antiviral. Besides several trials for pharmaceutical application of RA, it is currently used as a food and cosmetic ingredient. RA is currently produced by extraction from plants and chemical synthesis however, due to limited availability of plant sources and complexity of the chemical synthesis method, there is an increasing interest in producing RA by microbial fermentation

In this study, we aimed to produce rosmarinic acid by engineered baker's yeast *Saccharomyces cerevisiae*. Multiple biosynthetic pathway variants, carrying only plant genes or combination of plant and *E. coli* genes, were implemented. We show, for the first time, the applicability of a yeast host for rosmarinic acid production

Keywords: *Saccharomyces cerevisiae,* Rosmarinic acid, *p*-coumaric acid, Cytochrome P450 reductase

[§] Equally contributed

Identification and Characterization of the Novel ATF Genes Encoding Alcohol Acetyltransferases in *Saccharomycopsis fibuligera*

POSTER PRESENTATION ID: 166

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Aroma ester components, including isoamyl acetate and phenylethyl acetate, are responsible for the fruity character of fermented alcoholic beverages such as beer and wine. Acetate esters are produced by fermenting yeast cells via alcohol acetyltransferase-catalyzed intracellular reactions. The *ATF* genes, encoding alcohol acetyltransferases, in various yeast species have attracted intensive interest due to their biotechnological potential in aroma component production. By bioinformatics analysis of the whole genomes of amylolytic dimorphic yeast *Saccharomycopsis fibuligera* isolates from Nuruk (Choo et al., 2016), we identified 12 multiple *ATF* orthologues (*SfATF*) in the diploid genome of *S. fibuligera* KJJ81.

In order to investigate the enzymatic activity of the new searched alcohol acetyltransferases encoded by the *S. fibuligera ATF* orthologues, each of *SfATF* genes was expressed as a recombinant protein with His-tagging at its C-terminus in the heterologous host *Saccharomyces cerevisiae atf1 atf2* deletion mutant. The expression of *SfATF* was directed under the *TEF1* promoter and analyzed by western blotting using anti-His antibody. The volatile ester production was analyzed by headspace GC-MS.

The newly identified *SfAtf* proteins, encoded by 12 multiple *SfATF* genes, display quite low sequence identities to *S. cerevisiae ATF1* from 13.3 to 27.0 %. All of them, except *SfAtf(A)4p and SfAtf(B)4p*, contain the activation domain (HXXXD) conserved in other Atf proteins. Western blot analysis showed that *SfATF(A)2*, *SfATF(B)2* and *SfATF(B)6*, which showed relatively high mRNA expression in the native host under normal culture condition, were stably expressed in *S. cerevisiae*. Analysis of the fermentation products confirmed that the *S. cerevisiae* strains expressing *SfATF(A)2*, *SfATF(B)2* and *SfATF(B)6* showed a high-level production of isoamyl acetate and phenylethyl acetate. Especially, the *S. cerevisiae* strain expressing *SfATF(B)6* showed additional ester production of butyl acetate, indicating the differences in substrate specify of the *SfAtf* proteins.

In this study, we presented the novel *ATF* genes from *S. fibuligera*, *SfATF(A)2*, *SfATF(B)2* and *SfATF(B)6*, encoding functional alcohol acetyltransferases. The newly isolated *ATF* genes with diverse substrate specificity are expected to be usefully exploited for the enhanced production of several flavor ester compounds.

Keywords: Aroma components, Acetate esters, Alcohol acetyltransferases, *ATF*, *Saccharomycopsis fibuligera*

Acknowledgement: The work was supported by the National Research Foundation of Korea, Grant No. NRF2018R1A5A1025077 (Advanced Research Center Program), and by the Korean Ministry of Agriculture, Food and Rural Affairs, Grant No. 918010042HD030 (Strategic Initiative for Microbiomes in Agriculture and Food).

Establishing an Analytical Method for Determination of Carotenoid Compounds Extracted from *Rhodotorula toruloides* and *Rhodotorula babjevae*

POSTER PRESENTATION ID: 174

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Carotenoids belong to the most widespread natural colouring agents. Some yeast strains contain essential micronutrients and potent natural anti-oxidants. The main objective of this study was to investigate the carotenoid content and composition in two oleaginous yeasts, *Rhodotorula toruloides* and *Rhodotorula babjevae*.

The oleaginous yeasts were grown in shake flasks on lignocellulose hydrolysate for 4 days and the cells were then harvested and freeze-dried. Traditional acetone-extraction method was adopted for the extraction of the carotenoids from the freeze-dried yeast cells. The extracted samples were subsequently analysed by Ultra High Performance Liquid Chromatography (UHPLC). The UHPLC method was optimised and validated for eight carotenoids: α-, β-, γ-, δ-, ε-carotene, astaxanthin, canthaxanthin and lycopene for linearity, precision and recovery.

A number of carotenoids were identified in the yeasts, including β- and γ- carotene. We also found that some triglycerides were co-extracted with the acetone method, which may question earlier reports on carotenoid amounts produced by red oleaginous yeasts.

We established methods that will make it possible to investigate and optimise carotenoid production in red yeasts. The optimised analytical method will be interesting not only for carotenoid determination in microbes, but also for food industry and aquaculture.

Keywords: Oleginous yeasts, Carotenoids, *Rhodotorula toruloides*, *Rhodotorula babjevae*

Acknowledgements: Our research was supported by The Swedish Research Council for Environment, Agricultural Sciences and Spatial Planning (Formas), Grant Numbers 213-2013-80 and 2018-01877. We acknowledge a FEMS-mobility grant to LO.

Utilization of Monosaccharides from Sugar Beet Pulp Hydrolysates Rich in Pectic-Oligosaccharides (POS)

POSTER PRESENTATION ID: 179

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The sugar industry is one of the most waste-producing brunch in the agri-food industry. Annually, hundreds of tonnes of waste biomass in the form of beet pulp are created. This biomass, can become a source of valuable compounds used in the chemical, and biotechnological industry.

The aim of this work was to check the suitability of selected yeast strains for the removal of monosaccharides from beet pulp hydrolysates rich in pectic-oligosaccharides (POS). The research material consisted of two kinds of hydrolysates rich in mono- di and trisaccharides (glucose, fructose, mannose, galactose, arabinose, raffinose, xylose). The tested hydrolysates were obtained by enzymatic hydrolysis (using cellulolytic and pectinolytic preparations at 45 ° C for 3 hours) - the process was carried in in a Parr Instrument Company reactor model 4552, (equipped with a temperature control system and mixing) in a such way, so that the oligosaccharides (13.8 g / 100 g dry weight - expressed as di-Ara) were present in the final product. These compounds may have a prebiotic effect, and be a valuable component for animal feed.

Yeast strains *Metschnikowia pulcherrima* NCYC747, *Scheffersomyces stiptis* NCYC1541, *Kluyveromyces marxianus* NCYC179, *Saccharomyces cerevisiae* TT ŁOCK105, *Saccharomyces cerevisiae* Tokay ŁOCK0204, *Kluyveromyces lactis* ŁOCK0028, *Metschnikowia sinensis* 1.4 TKC, *Saccharomyces cerevisiae* Ethanol Red - Leaf/Lesaffre Advanced Fermentation, *Saccharomyces cerevisiae* Lalvin ICV K1-V1116 Lallemand were cultured on hydrolysates. The saccharide concentrations were determined using dedicated biochemical assay kits from Megazyme. The optical density of the culture and the number of colony forming units were also determined.

Keywords: Carbohydtate utilization, Sugar beet pulp, Pectic-oligosaccharides

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Yeast Single Cell Protein Production from a Biogas Co-Digestion Substrate

POSTER PRESENTATION ID: 206

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Aquaculture will play an integral role in providing sustenance for the growing world population and is the world's fastest growing animal food producing sector. However, the growth of aquaculture will be limited by the availability of protein-providing feed ingredients, which are to a large part produced from dwindling wild fish stocks. Yeast-based single cell protein has been shown to be a viable alternative to fish meal in cultured fish diets that does not compete with human food interests. Financially viable production of fish feed is dependent on readily available and cheap substrate. In this study, we have evaluated the use of biogas co-digestion substrate, consisting of municipal solid waste and agricultural waste, as a substrate for the production of yeast biomass for use as a fish feed protein source.

Substrate was collected from a local biogas plant, and sterile filtered. Strains of *Wickerhamomyces anomalus*, *Pichia kudriavzevii*, and *Blastobotrys adeninivorans* were chosen based on suitable characteristics, such as metabolic versatility and phytase production. The substrate was chemically characterized before and after yeast cultivation. Yeast biomass was analyzed for gross nutritional and amino acid composition. Methane production from spent substrate was evaluated using a biomethanation potential assay to assess the differential impact of each yeast strain on downstream biogas production.

All strains grew well on the substrate, yielding 7.0–14.8 g/l biomass, with crude protein contents of 22.6–32.7%. Productivities ranged from 0.53–0.99 g/l/h. Compared to white fish meal, yeast biomass was deficient in methionine and arginine, but the commonly limiting essential amino acid lysine was present in levels comparable to fish meal. Biomethanation potential of the spent substrate was statistically significantly different depending on the yeast strain.

Biogas substrate is in many ways an ideal substrate for yeast single cell protein production as it is readily available, with mature logistics networks in place. The results of this study indicate that biomass produced from such substrate is suitable for inclusion in aquaculture feed formulations, and that the choice of strain will be important for downstream biogas production and overall yields.

Keywords: Single cell protein, Aquaculture nutrition, Biogas, *Wickerhamomyces anomalus, Pichia kudriavzevii, Blastobotrys adeninivorans*

Importance of *Yarrowia lipolytica* for Industrial Applications

POSTER PRESENTATION ID: 213

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The yeast *Yarrowia lipolytica* represents as an attractive tool for biotechnological applications. This yeast has recently attracted the attention in the industry with its own characteristic properties and ability to produce some important substances. It is able to produce especially lipids, single cell oils, extracellular enzymes and single cell proteins, organic acids such as citric acid, isocitric acid, α-ketoglutaric acid, pyruvic acid and succinic acid from various carbon sources under different conditions. In addition, it can carry out fatty acid production such as dihomo-γ-linoleic acid, eicosapentaenoic acid, docosahexaenoic acid and arachidonic acid besides bioconversions of environmental pollutants. Furthermore, it can be used for contribution to the organoleptic characteristics of dairy products. Therefore, it has been classified as Generally Regarded as Safe (GRAS) and also can be isolated easily from wide range of environment as meat, poultry, dairy product, soy sauce, sea product and polluted oil. They are obligate aerobic non-conventional yeasts, which tolerate high level of salt, low temperature and low pH. Due to existence of extracellular lipolytic and proteolytic enzyme activities of this organism, proteins and lipids can be degraded. The product can vary according to carbon source used.

Keywords: *Yarrowia lipolytica*, Metabolic engineering, Single cell oil, Microbial lipids, Industrial fermentation

Production of Tailor-made Enzymes for the Catalysis of 2,5-Furandicarboxylic Acid in *Blastobotrys (Arxula) adeninivorans*

POSTER PRESENTATION ID: 218

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2,5-Furandicarboxylic acid (FDCA) is a bio-based alternative to petrochemically produced terephthalic acid, which is mainly used in the formation of polyethylene terephthalate (PET), an important building block in the packaging and textile industries. Chemical production of FDCA, although it appears suitable for large-scale use, has disadvantages such as the high stoichiometric use of oxidants and the use of environmentally harmful and non-recyclable catalysts. For this reason, biotechnological production has become the focus of attention in recent years. In our project we focus on the targeted development of biological systems to generate tailor-made enzymes that catalyse all reaction steps from 5-Hydroxymethylfurfural (HMF) to FDCA in the yeast *Blastobotrys (Arxula) adeninivorans*.

Our approach uses secreted aryl alcohol oxidases (AAOs) that are known to use HMF as initial substrate in a cascade of subsequent oxidation steps. *AAO* genes of various fungal origins were identified, isolated and constitutively expressed in *B. (A.) adeninivorans* by the Xplor®2 transformation and expression platform allowing the introduction of the *AAO* expression module either as yeast rDNA integrative expression cassette (YRC) or as yeast integrative expression cassette (YIC) into the yeast genome. The activity of AAO was assessed by measuring the conversion of the test substrate Veratryl alcohol (3,4-Dimethoxybenzyl alcohol) to Veratraldehyde (3,4-Dimethoxybenzaldehyde), the formation of which causes a spectrometric absorption change. Since aryl alcohol oxidases are inherently weak in catalysing the final step in FDCA synthesis, we have additionally performed a mutagenesis-based approach aimed at obtaining a tailor-made enzyme with high efficiency in the synthesis of FDCA from HMF, which can be conveniently isolated from the fermentation broth.

We have successfully developed *B. (A.) adeninivorans* strains with an initial maximum aryl alcohol oxidase activity of 6.3 units per litre in the culture supernatant and achieved a significant increase in activity using the targeted mutagenesis approach. Our future work will focus on the final oxidation step, which will provide sufficient amounts of FDCA as an interesting substitute for terephthalic acid in the production of bio-based plastics.

Keywords: *Blastobotrys (Arxula) adeninivorans*, Bio-based plastics

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Esters Production by Non-*Saccharomyces* Yeasts During Alcoholic Fermentation

POSTER PRESENTATION ID: 223

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In recent years industrial ethanol production, using different raw materials as a source of sugars (residual biomass, such as forest, industrial, or municipal wastes) has increased. However, the major limitation for the production of second-generation ethanol is the high capital investment cost. Hence, integrated biorefineries could be implemented for the production of ethanol and by-products with high added value. Esters represent the largest group of aromatic components in fermented broth; and they provide the characteristic aroma of fruits, honey, and roses. Esters can be used as ingredients in food industry, tobacco, pharmaceuticals, agriculture, cosmetics, lubricants, and metal treatment. Esters are formed by the reaction between an alcohol and a carboxylic acid, and many factors influence their production during alcoholic fermentation, but the first factor is the yeast strain used. Therefore, the objective of the present study was the evaluation of the production of esters by different strains of non-*Saccharomyces* yeasts.

Five yeast strains were evaluated in this study, three different strains of *Pichia kluyveri* (MGL7, MGL3, GU3), *Kluyveromyces marxianus* (OFF1), and *Saccharomyces cerevisiae* Ethanol Red® (ERD) as control. Fermentations were carried in Erlenmeyer flasks containing mineral medium with 20 g/L of glucose as carbon source, at 30°C, 100 rpm for 24 hours. Ethanol and esters analyses were performed by gas chromatography.

All the non-*Saccharomyces* yeasts were able to produce ethanol and esters (ethyl acetate, isoamyl acetate, and phenyl acetate) under assay conditions. However, *P. kluyveri* GU3 was the highest producer of ethanol (9.16±0.26 g/L) and esters; being ethyl acetate the majority ester produced by GU3 with 66.84±8.21 mg/L, followed of isoamyl acetate (8.55±0.48 mg/L) and phenyl acetate (8.97±0.41 mg/L). In contrast, *S. cerevisiae* only produced ethanol (8.86±0.87 g/L), ethyl acetate (1.72±0.27 mg/L) and phenyl acetate (3.51±0.15 mg/L).

We demonstrated that non-*Saccharomyces* yeasts have the ability to produce high value-added metabolites such as esters in addition to producing ethanol at concentrations similar to *S. cerevisiae*; so, they can be interesting yeasts for use in a bio-based biorefinery.

Keywords: Esters, non-*Saccharomyces* yeast

Acknowledgements: Thanks to CONACYT for project FSE 250014

Production of Gold Nanoparticles by Yeasts and Their Antibacterial Activity Against *E. coli*

POSTER PRESENTATION ID: 224

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The synthesis of nanoparticles by biological methods has gained importance because it reduces ecological damage to the environment and or costs, when compared to the methods currently used for their production.

In this work, two yeasts were used to produce gold nanoparticles of different sizes (by biological reduction of a gold salt); and the morphologies and antibacterial activity of gold nanoparticles produced were evaluated against *E. coli* bacteria.

Three morphologies of gold nanoparticles were obtained varying the yeast culture conditions; and among them, two morphologies were effective against the bacteria studied.

Keywords: Gold nanoparticles, Yeast

Acknowledgements: Thanks to CONACYT for project CB 237737 and to Dr. Miguel Avalos and Héctor Gabriel Silva Pereyra from LINAN IPICYT for micrographies.

Yeasts as Biocontrol Agents

Characterization of the Growth's and Phenyllactic Acid Production by the Yeast *Geotrichum candidum* to Use It as a Biocontrol Agent in the Brewing Process

POSTER PRESENTATION ID: 153

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Geotrichum candidum is a filamenteous yeast recognized as safe, and that already shown its ability to inhibit the growth of pathogenous microoganisms. It's use in bioprocess appears usefull for the natural bioprotection of the products.

In the brewing process, *G. candidum* is used as a biocontrol. Our previous laboratory works showed its ability to reduce concentration of mycotoxins T2 produced by *Fusarium langsethiae*, but the mechanism(s) of action was/were steel unknown.

New experiments on others *Fusarium* species shows that *G. candidum* can reduce T-2 toxin produced by *F. langsethiae* 2297 *and F. sporotrichioides* up to 14 times and 9 times, respectively, within three days.

Thanks to sequential cultivation, co-cultures cultivation and biochemistry analysis, a potential metabolite was identified and seemed to be linked to the reduction of T2-toxin concentration in the medium where *Fusarium* species were cultivated.

Finally, in order to have a best comprehension of the *G. candidum* growth and it's metabolite production, several media of cultivation were tested such as YM, Malt Extract Broth, and a synthetic media and and differents conditions of cultivation with various shaking speeds consisting of 0 rpm, 150 rpm, 200 rpm and 250 rpm were also experimented. The results shows that it seems to be important to work under agitated conditions in order to promote the growth of *G. candidum*, and it's metabolic activities.

Keywords: *Geotrichum candidum*, *Fusarium langsethiae* 2297, *Fusarium sporotrichioides*, T-2 mycotoxin

Acknowledgements: Thanks to ANR for funding

Effect of Myclobutanil Pesticide on the Biochemical Behaviour of a Novel *Saccharomyces cerevisiae* Strain During Non-Aseptic Alcoholic Fermentation

POSTER PRESENTATION ID: 239

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Myclobutanil is a chiral triazole pesticide (fungicide) that is employed worldwide to control various fungal diseases that may occur in grapes, cereals, and in general fruits and vegetables. Although its acute toxicity is low, and it is known to be almost inactivate in must, myclobutanil has been reported to increase liver mixed function oxidase, disrupt steroid hormone homeostasis, and cause hepatocyte hypertrophy and testicular atrophy in rodents. As a result, its effect needs to be eliminated in produced wines.

In the present work the yeast strain *Saccharomyces cerevisiae* LMBF-Y 16 (Rhône), was studied regarding its biochemical behavior (biomass production, substrate uptake, ethanol and glycerol biosynthesis) and its ability to remove myclobutanil pesticide from the culture medium during fermentation. Grape must in which myclobutanil was added in various concentrations was used, and trials were carried out in agitated flasks.

The strain showed high fermentative efficiency in parallel with high ethanol production which in some cases achieved values close to the maximum theoretical ethanol yield (0.49 g of ethanol per g of sugar). At grape musts in which commercial glucose and fructose were added (initial total sugars at *c*. 250 g/L) very high ethanol concentrations (i.e. ranging between 105 and 112 g/L) were reported irrespective of the addition of myclobutanil into the medium. Moreover, there was observed a significant myclobutanil removal during fermentation (5-27%) as a result of the adsorptive or degradative capacity of the yeast. Myclobutanil addition showed no effect on the cellular growth. On the other hand, high initial sugar concentration and produced ethanol quantities had a preventative effect on cellular growth.

These results are considered very promising as novel yeast strains can be applied for high ethanol production providing a parallel pesticide reduction in a general biorefinery concept with many environmental benefits.

Keywords: *S. cerevisiae*; biochemical behavior; alcoholic fermentation; myclobutanil.

Acknowledgemen: The current investigation was financially supported by the project entitled “Exploitation of new natural microbial flora from Greek origin amenable for the production of high-quality wines” (Acronym: Oenovation, project code T1EΔK-04747) financed by the Ministry of National Education and Religious Affairs, Greece (project action: “Investigate – Create – Innovate 2014-2020, Intervention II”).

Lipid Production by *Rhodosporidium toruloides* Growing on Renewable Carbon Sources in Batch and Fed-batch Cultures

POSTER PRESENTATION ID: 241

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Yeast lipids present increasing interest as alternative non-food feedstocks for biodiesel production, therefore, this study was focused on enhancing lipid production by the yeast *Rhodosporidium toruloides*, strains DSM 444 and NRRL Y-27012 cultivated on renewable resources employed as microbial substrates. Fermentations were carried out under nitrogen-limited conditions using 50 g/L of different carbon sources, namely xylose and crude glycerol. Xylose was used as carbon source in order to mimic the principal waste-stream originated from paper production facilities (viz. the spent-sulfite liquor). On the other hand, crude glycerol is the main by-product of the industrial production of biodiesel.

The effect of sodium lignosulfonate (SL), a paper industry by-product, on cell growth and lipid production by the yeast cultivated on xylose-based media was explored. Both strains were shake-flask cultured under nitrogen-limited conditions using xylose (at 50 g/L), while SL was added at varying concentrations. Fermentation of the strain NRRL Y-27012 was further carried out in a fed-batch bioreactor with optimum SL addition. Moreover, the effect of NaCl addition on lipid production by the two strains grown on crude glycerol was also investigated.

Maximum lipid production of 4.77 g/L by the strain DSM 4444 was obtained in xylose-based media supplemented with 20 g/L SL, while in NRRL Y-27012 strain, maximum lipid production of 5.30 g/L was obtained after the addition of 10 g/L SL. In fed-batch bioreactor experiments carried out with the strain NRRL Y-27012, lipid production of 17.0 g/L (corresponding to 29.7 g/L of DCW) was achieved. The yield of lipid produced per unit of xylose consumed was ≈0.19 g/g. When the strain NRRL Y-27012 was cultured in crude glycerol-based media the produced intracellular lipid was up to 8.23 g/L, while the addition of NaCl had a negative effect upon the process of lipid production. Fatty acid composition of cellular lipids revealed increased concentrations of oleic acid in all trials.

The findings in this study showed that the yeast can produce high amount of lipids rich in oleic acid, constituting perfect materials amenable to be converted into “2nd generation” biodiesel using low cost substrates.

Keywords: Glycerol, Lipid Production, *Rhodosporidium toruloides*, Xylose

Acknowledgement: The current investigation was funded by the project entitled “Adding value to biodiesel-derived crude glycerol with the use of Chemical and Microbial Technology” (Acronym: Addvalue2glycerol, project code T1EΔK-03002), financed by the Ministry of National Education and Religious Affairs, Greece (project action: “Investigate – Create – Innovate 2014-2020, Intervention II”).

Value-added Compounds by a *Yarrowia lipolytica* Strain Growing on Biodiesel-derived Glycerol Diluted with Olive-mill Wastewaters

POSTER PRESENTATION ID: 242

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One of the major environmental problems is the high toxic agro-industrial waste, olive mill wastewater (OMW), deriving from olive oil production. On the other hand, the continuous development of biological liquid fuel industry (biodiesel) makes it mandatory the process and exploitation of the increased production of its main by-product, called biodiesel-derived glycerol or crude glycerol. *Yarrowia lipolytica* yeasts have been reported to be capable to produce a plethora of value-added compounds during growth on several types of agro-industrial co-products, while crude glycerol has been reported as a great substrate for microbial growth. In the present investigation, crude glycerol at initial concentration of 70 g/L was blended with OMWs (yielding in a liquid medium containing c. 2 g/L of total phenolic compounds), which partially replaced tap water, performing fermentations by *Y. lipolytica* ACA-YC 5031. The purpose of this study was the simultaneous detoxification of OMW and production of value-added compounds.

Batch culture fermentations were carried out under nitrogen-limited conditions at different salt (NaCl) concentrations (1%, 3%, 5% w/w) added. Dry weight biomass, intracellular polysaccharides (IPS) and intracellular lipids were determined, while glycerol removal, citric acid and polyols secretion were monitored using high-performance liquid chromatography. The intracellular fatty acid composition was determined using gas chromatography, while removal of phenolic compounds and color were also monitored.

In a glycerol-based medium blended with OMWs, remarkable DCW and IPS production (8.4 g/L and 2.6 g/l, respectively) were observed. Mannitol values reached 8.8 g/L while erythritol reached 4.9 g/L. Addition of salt reduced the produced biomass, while the IPS production was almost the same during the fermentations. Increasing the salt concentration resulted in decreased biomass and polyols, while citric acid was significantly increased reaching a final value of 54.0 g/L (conversion yield on glycerol consumed =0.88 g/g) in the trial with addition of 5% NaCl. Finally, significant color and phenols removal were observed, evaluating the yeast as a decontamination medium for the OMW.

The results indicated that the strain *Y. lipolytica* ACA-YC 5031 is a great candidate for the production of value-added compounds with biotechnological interest, and also for the bioremediation of OMWs. The selected conditions and substrates promoted the production of value-added compounds in high concentrations using low cost, renewable, substrates.

Keywords: Glycerol, Olive-mill wastewater, Value-added compounds, *Yarrowia lipolytica*

Acknowledgement: The current investigation was funded by the project entitled "Adding value to biodiesel-derived crude glycerol with the use of Chemical and Microbial Technology" (Acronym: Addvalue2glycerol, project code T1EΔK-03002), financed by the Ministry of National Education and Religious Affairs, Greece (project action: "Investigate – Create – Innovate 2014-2020, Intervention II").

Yeast Taxonomy, Ecology and Biodiversity

Growth in Spent Sulphite Liquor and Biotransformation of Vanillin by Yeasts from Decaying Wood

POSTER PRESENTATION ID: 161

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Lignocellulosic biomass is a challenging ecological niche for microorganisms. Spent sulphite liquor (SSL), which derives from acid hydrolysis of lignocellulosic biomass, is an even greater challenge, due to the presence of toxic phenolic compounds, specific monosaccharides, lignosulphonates and inhibitors, such as HMF, furfural, formic acid and acetic acid. One of these inhibitors is vanillin, a lignin monomeric derivative. Could yeasts that originate from wood tolerate vanillin and grow in the presence of SSL? A basidiomycetous yeast, *Cystobasidium laryngis* strain FMYD002, grew in vanillin-supplemented media, and biotransformed vanillin into vanillyl alcohol. It is part of a collection of yeasts isolated from decaying wood on the Faroe Islands. The aim of the present study was to determine the vanillin biodegradation profiles and the ability to grow in the presence of SSL.

These yeasts were identified by ITS1–5.8S–ITS2 and D1/D2 sequence homology. The relationship between wood-habitat and vanillin tolerance by cultivating the yeasts in the presence of 1 mM vanillin. The vanillin biodegradation profiles were determined by LC-MS, using the standards: vanillin, vanillyl alcohol and vanillic acid. The growth in different concentrations of SSL was evaluated.

Strains of *Cystobasidium laryngis*, *Cystofilobasidium infirmominiatum*, *Goffeauzyma gastrica*, *Goffeauzyma* sp., *Naganishia* sp., *Holtermanniella* sp., *Rhodotorula* sp., *Nadsonia starkeyi-henricii*, *Debaryomyces hansenii*, *Debaryomyces* sp., *Candida sake* and *Candida argentea* were identified. Most strains were able to grow in vanillin-supplemented medium. The predominant biodegradation product was vanillyl alcohol followed by vanillic acid. Several other biodegradation products were detected. Most strains were able to grow in the presence in SSL. Species of *Candida* and *Debaryomyces* were most tolerant, whereas species of *Nadsonia*, *Holtermanniella* and *Naganishia* grew poorly.

Many of the yeast species described herein are associated with wood or cold environments. Ability to grow in the presence of vanillin did not completely correlate with tolerance to SSL. However, the strains that grew at the highest concentration of SSL also grew well in the presence of vanillin, from which they rapidly produced large amounts of vanillyl alcohol, and many other biodegradation products. Conversely, the isolates with poor or no growth in vanillin had extremely low or no tolerance to SSL. Thus, high tolerance to vanillin appeared to be a prerequisite for growth in SSL-based medium. Different yeasts have tolerance to different inhibitors present in SSL. A comprehensive analysis of growth and biodegradation of vanillin produced five groups, containing specific yeast genera.

Keywords: Lignin, SSL, Spent Sulphite Liquor, Biodegradation

Molecular-Genetic Polymorphism of Beta-Galactosidase LAC Genes of the Dairy Yeast *Kluyveromyces lactis*

POSTER PRESENTATION ID: 187

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Dairy yeasts *Kluyveromyces lactis* are able to ferment lactose due to the presence of the beta-galactosidase enzyme. Polymeric *LAC* loci have a complex structure and consist of two closely linked structural genes *LAC4* (beta-galactosidase) and *LAC12* (lactose permease), and a regulatory sequence (Dickson & Riley 1989; Fairhead & Dujon 2006; Naumov 2008; Naumov & Naumova 2014).

Using molecular karyotyping and Southern hybridization, we have conducted a large-scale molecular-genetic screening of *Kluyveromyces lactis* strains isolated from different dairy products and natural sources in ex-USSR, Europe, North America, and New Zealand. Recombination and complementation analyses were done as described in Naumov (2008).

Molecular karyotypes of strains studied being general similar, some length polymorphism of chromosomes II and III was observed. Hybridization with the *LAC4* probe revealed the presence of the *LAC1* locus in seven strains, *LAC2* in 19 strains and *LAC3* in four strains. Polymeric loci *LAC1/LAC2* (Y-328, Y-492) and *LAC1/LAC3* (Y-1868) were found in three strains. Most of dairy strains had the *LAC2* locus. There was no correlation between the geographical origin of the strains and the presence of a certain *LAC* loci. According to the complementation analysis, Lac-negative strains of *K. lactis* are not able to ferment lactose due to the absence of active *LAC4* и *LAC12* genes. Southern hybridization of chromosomal DNA with the *LAC4* and *LAC12* probes revealed no hybridization signals and, therefore, absence of even silent corresponding sequences in these strains. Phylogenetic analysis showed significant differences between the *LAC4* proteins of the Lac^+ *K. lactis* strains and the corresponding proteins of the yeasts *Scheffersomyces*, *Sugiyamaella* and *Debaryomyces*.

Screening of the *LAC4* genotypes in different *K. lactis* strains revealed three polymeric *LAC* loci: *LAC1* (chromosome III), *LAC2* (chr. II), and *LAC3* (chr. IV), each of which includes two closely linked structural *LAC4* genes (beta-galactosidase) and *LAC12* (lactose permease). The evolution of beta-galactosidase *LAC* genes of ascomycetous yeasts is discussed.

Keywords: *Kluyveromyces lactis*, *LAC4-LAC12* cluster, Genetic hybridization, Phylogenetic analysis

Acknowledgement: The study was supported by the Russian Foundation for Basic Research (project no. 18-54-52002).

Investigating the Grape Fungal Community Structure Across Vintages and Along Regional Geographical Scales

POSTER PRESENTATION ID: 207

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Microbial community structures associated with wine grapes and fermentation play a critical role in grape and wine quality. Furthermore, they are important determinants of wine aroma and wine typicity. However, the grape microbiota may vary significantly amongst geographic sites and change with vintage. The current study aimed to unravel the fungal community composition of Cabernet Sauvignon grape must from three vineyards located in three distant areas within the Stellenbosch wine region and identify the core resident fungal genera.

Temporal sampling (2017 and 2018) was performed. A systematic sampling design was applied to collect 30 kg of grapes from each vineyard. The grapes were mechanically crushed and samples withdrawn from the fresh must and used for DNA extraction and subsequent amplification of the ITS-5.8S rRNA gene region. Fungal community composition was evaluated with Illumina Amplicon sequencing followed by data processing on the QIIME pipeline. Spontaneous fermentations were performed and chemical analysis of the wines was performed with GC-MS.

PCoA plots showed that the grape fungal community of the two vintages were clearly segregated. ANOSIM revealed that the overall fungal communities were similar with significant overlap. However, it was evident that vintage had a slightly stronger influence on fungal community variations than region of isolation. The *Ascomycota* were the most dominant fungal group present in grape must, and species of the genera *Candida*, *Hanseniaspora*, *Lachancea*, *Pichia*, *Metschnikowia* that typically constitute the wine yeast consortium, were identified in all samples albeit at varying relative abundances. Evidently, these variations were sufficient to produce wines with distinct chemical signatures.

The interest in regional grape microbiome has gained impetus in the past five years as winemakers try to identify ways to produce unique wines that better express the terrior. Our study begins to shed light on microbial signatures in South African vineyards and will make valuable contribution in this field of research.

Keywords: Microbiome, Biogeography

Acknowledgements: This work was supported by the Wine Industry Network for Expertise and Technology (Winetech), grant SU IWBT 16/02, the National Research Foundation-Technology and Human Resources for Industry Programme (grant number TP14080184824)

Microbiome and Metabolic Profiles from Two Syrah Vineyards in Portugal

POSTER PRESENTATION ID: 208

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Wines made from identical grape cultivars but grown in different regions are appreciated for their distinctive features. The identification of regional-specific microbiota has recently gained much interest, especially with the use of high-throughput DNA sequencing that has brought new insights on microbial diversity. The putative relation between grape microbiome and wine metabolic profiles was the main purpose of this undergoing project starting in the 2018 wine vintage. In particular, the study focused on comparative data of Syrah grape variety cultivated in two vineyards in the wine Demarcated Region of Lisbon (Portugal).

The maturation of Syrah grapes cultivated in two closely located vineyards was controlled with physicochemical analysis up to harvest. Grape berries were randomly selected at day of harvest and must samples collected through fermentation (after crushing, mid- and end-fermentation) and immediately frozen at -80°C. Microvinifications (50-60L) were performed in duplicate under the same conditions and progress was monitored by daily measurement of density and temperature after homogenization. Different DNA extraction methodologies were tested and adapted to each sample type. Must and wine microbiome was analyzed based on the 4th NGS Oxford Nanopore technology and metabolite profiles were obtained by LC-MS/MS (Elute-QTOF).

The generated sequencing data of whole grapes submitted to the WIMP workflow for taxonomic classification allowed the detection of over 40 genera. Most of the genera detected were similar, the genus *Clavispora* accounting for the majority of the reads, but diversity and relative abundance differences were observed in between the two vineyards. Differences in metabolic profiles namely in sugars and phenylpropanoids were also noticed for grape as well as for must fermentation samples.

Keywords: Microbiome, Metabolite, Syrah, Grape, Wine

Acknowledgements: Work supported by UID/MULTI/04046/2019 Research Unit grant from FCT, Portugal (to BioISI) and PO Centro-04-3928-Feder-000001.

Indigenous Non-*Saccharomyces* Yeast Diversity During Spontaneous Fermentation of 'Karalahna' and 'Cabernet Sauvignon' Grapes

POSTER PRESENTATION ID: 215

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Çanakkale is one of the most important regions in Turkey for winemaking with a variety of local and international grapes. In the present study, isolation and molecular identification of indigeneous non-*Saccharomyces* yeasts during spontaneous fermentation of Karalahna (KL) and Cabernet Sauvignon (CS) grapes which are grown in Bozcaada and Eceabat districts of Çanakkale were performed, respectively.

The molecular identification of the yeast strains isolated from samples was carried out by using Restriction Fragment Length Polymorphism (RFLP) method. Yeast isolates were identified by PCR amplification of the internal transcribed spacers between the 18S and 26S rDNA genes (ITS1-5.8S-ITS2) and subsequent restriction analysis (*Cfo*I, *Hae*III and *Hinf*I). Isolation and identification of yeasts during spontaneous fermentation of KL and CS grapes were evaluated at the beginning (alcohol content 1%), middle (alcohol content 4%) and end of the fermentation (alcohol content 9%).

138 of the isolates were identified as yeast and further molecular analysis were applied in these isolates. *Candida albicans* (as dominant flora), *Zygosaccharomyces bisporus*, *Dekkera anomala* and *Issatchenkia terricola* were isolated at the beginning of the alcoholic fermentation of KL grapes. *Candida albicans* and *Dekkera anomala* were found in the middle and the end of the fermentation of KL grapes. On the other hand, *Dekkera anomala* was identified as dominant flora at the beginning of the fermentation of CS grapes. *Dekkera anomala*, *Candida apicola* and *Candida albicans* were isolated during the spontaneous fermentation of CS grapes.

This is the first study reporting the yeast flora of KL wines during spontaneous alcoholic fermentation. Recently, it is revealed that non-*Saccharomyces* species which is naturally found in grapes have great potential for winemaking. The yeast strains identified in this work need to further investigations for their oenological properties.

Keywords: Spontaneous fermentation, Yeast, Karalahna, Cabernet Sauvignon

Acknowledgements: This study was supported by the Scientific and Technological Council of Turkey (Project No: 117O313).

New Trends in Winemaking: The Effects of Non-*Saccharomyces* Yeasts on Wine Aroma

POSTER PRESENTATION ID: 216

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Yeasts play a crucial role in wine fermentation and contribute to the sensory characteristics of wine. *Saccharomyces cerevisiae* is the main yeast responsible for the alcoholic fermentation of grape must. However, there is a complex microflora in spontaneous or natural fermentation of wine. Different strains of *Saccharomyces* and non-*Saccharomyces* yeasts are naturally present in wine fermentation derived from grapes, vineyards and the equipments used in winemaking.

It has been widely revealed that non-*Saccharomyces* species dominate the early stages of alcoholic fermentation and some of these yeasts are capable of surviving at advancing phases. In recent years, non-*Saccharomyces* yeasts have received great attention due to their positive contributions to the organoleptic properties of wine by their secondary metabolites such as higher alcohols, esters, acids, volatile thiols and extracellular enzymes. Therefore, there is a growing interest in isolation and characterization of non-*Saccharomyces* yeasts. Among these yeasts, *Candida, Hanseniaspora, Kloeckera, Zygosaccharomyces, Schizosaccharomyces, Saccharomycodes, Torulaspora* and *Pichia* genera are known as prevalent yeasts found on grapes and at the early stages of the fermentation process. Their application in winemaking especially sequential inoculation with *S. cerevisiae* is increasingly popular since they improve wine flavor and aroma composition.

This review focuses on the applications of non-*Saccharomyces* yeasts in winemaking, their effects on wine quality and future perspectives. We summarized the latest researches conducted on the selection of non-*Saccharomyces* yeasts as starter cultures for the production of typical wines.

Keywords: Wine, Aroma, Non-*Saccharomyces*, Fermentation

Yeast Diversity in Arbutus Unedo Fruits Fermentations

POSTER PRESENTATION ID: 222

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The arbutus tree (*Arbutus unedo* L.) is a forest species present in most of the Portuguese continental territory. The mature fruits are normally collected in autumn, during a period of 2-3 months, mostly used for the production of a distilled beverage made from fermented fruits. Traditionally, fermentation occurs due to wild microbiota, and without temperature control. The spontaneous fermentation can last for several months depending on weather temperature and humidity. Microbiota was analyzed at two sampling times and yeast isolates were identified.

Arbutus unedo fruits mid-fermentation samples were collected in duplicate from 2 different producers and placed in sterilized fermenting flasks at 16.5 ºC until end of fermentation. Sample weight lost was controlled during 2 months and plate count analyses (total count, yeast and acetic bacteria counts) were performed at time of collection and after 2 months. Twenty yeast colonies were isolated at each sampling time from both samples of producers 1 and 2, streaked to obtain pure culture and stored at -80 °C in glycerol before identification methods were performed. Isolates were analyzed using *Saccharomyces cerevisiae* specific PCR amplification with SC1 primer pair which generated a 301 bp product. The negative isolates for SC1 were further identified by sequence analysis of the region D1/D2 of the 26S rRNA gene.

A total of 160 isolates were analysed and the majority were positive for *S. cerevisiae*. Sequencing allowed detecting spicies such as *Saccharomyces bayanus, Lachancea cidri, L. thermotolerans* or even *Brettanomyces bruxellensis.* Samples from producer 1 revealed the presence of *S. cerevisiae, Saccharomyces bayanus and Brettanomyces bruxellensis* at mid-fermentation, while only *S. cerevisiae* was detected at the end of the fermentation. Otherwise samples from producer 2 enabled the detection of different species such as *Zygosaccharomyces bailii, Saccharomyces ludwigii* as well as *B. bruxellensis* at the end of fermentation, where the representatives of *S. cerevisiae* decreased. The influence of these species, usually associated with spoilage, in *Arbutus unedo* fermentations has to be clarified.

Further investigations into *S. cerevisiae* isolates will be carried out to uncover domestication events that enabled to cope with particular conditions of *Arbutus unedo* fermentations.

Keywords: Yeast, Diversity, Fermentation, *Arbutus unedo*

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Taxonomic Classification of Novel Yeast Species Isolated from Soil in Korea

POSTER PRESENTATION ID: 225

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Soil ecosystems were regarded as a reservoir for yeast and possessed extraordinary adaptations that allow them to survive in a wide range of environmental conditions. We analyzed the diversity of yeasts from the soil samples collected in Pocheon, Korea.

Around 400 strains were isolated, identification of species was made with sequence analysis. Phylogenetic analysis based on the D1/D2 domains of the large subunit rRNA gene and the internal transcribed spacer (ITS) regions and pairwise sequence analysis resulted in identification 400 isolates belonging to 31 different genera.

Among the 31 genera, *Cryptococcus* sp., *Cystofilobasidium* sp., *Filobasidium* sp., *Hannaella* sp., *Leucosporidium* sp., *Mrakia* sp., *Naganishia* sp., *Papiliotrema* sp., *Rhodotorula* sp., *Sampaiozyma* sp., *Solicoccozyma* sp., *Tricellula* sp. and *Vishniacozyma* sp. are the most abundant genera and the minor genera are *Aureobasidium* sp., *Bullera* sp., *Candida* sp., *Curvibasidium* sp., *Debaryomyces* sp., *Dioszegia* sp., *Holtermanniella* sp., *Meyerozyma* sp., *Mrakiella* sp., *Phaeotremella* sp., *Pseudeurotium* sp., *Rhodosporidiobolus* sp., *Rhodosporidium* sp., *Saitozyma* sp., *Sakaguchia* sp., *Sporobolomyces* sp., *Trichosporon* sp., *Udeniomyces* sp., and *Yarrowia* sp. Therefore, *Basidiomycetes* dominated with about 60% above the members of the *Ascomycetes*.

Keywords: Taxonomy, Novel yeast

Acknowledgements: This work was supported by a grant from the National Institute of Biological Resources (NIBR), funded by the Ministry of Environment (MOE) of the Republic of Korea (NIBR201928201).

Novel Yeast Species Isolated from Gut of Earthworm

POSTER PRESENTATION ID: 226

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A novel ascomycetous yeast species in the genus *Wickerhamomyces* was isolated from earthworm gut. Phylogenetic analyses using combined sequences of the small ribosomal subunit and the D1/D2 domains of the LSU, as well as the internal transcribed spacer regions, suggested that these strains belong to the *Wickerhamomyces* clade and that together they form one strongly supported phylogenetic clade. Pairwise sequence alignment of D1/D2 sequences in the GenBank (http://www.ncbi.nlm.nih.gov) database revealed that the novel species is related most closely to *Wickerhamomyces ochangensis*.

The novel species reproduced asexually; no sexual reproduction could be found. Growth was observed at 25 °C. Morphologically, the strains produced white-colored yeast colonies, with cells that were ovoid to ellipsoidal, making branched, true hyphae and pseudohyphae. Ascospore formation was not observed. Differences in their sequences, biochemistry, and morphology suggest they are representatives of distinct species of the genus *Wickerhamomyces*.

Therefore, the name *Wickerhamomyces albus* f.a., sp. nov. is proposed to accommodate the novel strains; the type strain is YA45^{T}.

Keywords: Taxonomy, Novel yeast

Acknowledgements: This work was supported by a grant from the National Institute of Biological Resources (NIBR), funded by the Ministry of Environment (MOE) of the Republic of Korea (NIBR201928201).

Isolation and Characterization of Novel Yeast from Soil in South Korea

POSTER PRESENTATION ID: 227

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A novel isolate belonging to the basidiomycetous genus *Cystofilobasidium* were obtained from soil sample collected from Pocheon, South Korea. Sequence analysis of the D1/D2 domains of the LSU rRNA gene showed that the novel species belongs to the *Cystofilobasidium* clade and is phenotypically and genetically divergent from currently recognized species in this clade.

The novel species reproduced asexually; no sexual reproduction could be found. Growth was observed at 20-25 °C. The growth of the novel yeast species is limited by its ability to metabolize only a few carbon and nitrogenous compounds. Morphologically, the strain produced red-pink colored yeast colonies, with cells that were ovoid to ellipsoidal on YM agar at 25 °C, 3 days. Differences in the sequence analysis, biochemical test results and morphology suggested that the isolate are representatives of distinct species of the genus *Cystofilobasidium*.

Therefore, the name *Cystofilobasidium rubrum* f.a., sp. nov. is proposed to accommodate the novel strain.

Keywords: Taxonomy, Novel yeast

Acknowledgements: This work was supported by a grant from the National Institute of Biological Resources (NIBR), funded by the Ministry of Environment (MOE) of the Republic of Korea (NIBR201928201).

Yeasts in Health and Probiotics

Using Yeast-Based-Model to Identify Drugs for VPS13-Dependent Rare Neurodegenerative Diseases

POSTER PRESENTATION ID: 103

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Neurodegenerative diseases are growing problem in aging society. A lot of efforts are put on research to understand major age-related neurodegenerative diseases, such as Alzheimer or Parkinson, although no disease-modifying drugs are available. There are also many rare and ultra-rare neurodegenerative diseases, including those dependent on mutations in *VPS13A-D* genes; chorea-acanthocytosis, Cohen syndrome, early-onset Parkinsonism and spinocerebellar ataxia. These diseases are much less studied and the cure is not available. *VPS13* genes are conserved from yeast to humans. Thus, yeast is a good model system to study function of Vps13 proteins, the effect of human mutations on cell physiology, and to screen for genetic and chemical suppressors of *vps13* mutations. In yeast, there is a unique *VPS13* gene and its deletion (*vps13*Δ) impairs intracellular trafficking, the actin cytoskeleton organization, the maintenance of mitochondrial DNA and other functions. Recently, we discovered that *vps13*Δ cells are hypersensitive to sodium dodecyl sulphate (SDS), commonly used detergent. We found this novel and simple growth phenotype very useful in genetic screen for multicopy suppressors of *vps13*Δ mutation, which we recently published.

Here, we used SDS-hypersensitivity phenotype and filter assay for identification of chemical suppressors of *vps13*Δ growth defect. We performed a drug screen using Prestwick Chemical Library, a collection of 1280 chemical compounds, most of which are accepted for use in humans. Western blot analysis and fluorescence microscopy were used to test effect of drugs on some phenotypes.

Based on the screen results and literature, we selected 7 substances for further research. After validation, we analyzed impact of these substances on other phenotypes of *vps13*Δ mutant, such as canavanine hypersensitivity, impaired transport of Sna3 protein to the vacuole, mitochondrial DNA escape and impaired actin cytoskeleton organization.

Our work allowed selecting drugs with the highest therapeutic potential for studies with the use of cell lines. Our findings may contribute in future to discovery of an effective therapy for diseases associated with *VPS13* genes.

Keywords: *vps13*Δ yeast model, Rare neurodegenerative diseases

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Drilling Beyond the Tip of the Iceberg: A Deeper Look at The Polymicrobial Interaction Between *Candida albicans* and *Pseudomonas aeruginosa* Using RNAseq

POSTER PRESENTATION ID: 111

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In past decades it has become evident that infection by clinically relevant microorganisms is rarely accomplished alone but, is the product of complex interaction and cross-talk between pathogens. This sparked the in-depth study of clinically significant microorganisms such as the dimorphic yeast *Candida albicans* and the bacterium *Pseudomonas aeruginosa* and the interaction between them. *In vitro* studies have provided evidence of an antagonistic interaction between the yeast and bacterium. However, a large amount of information is still needed to fully elucidate the interaction and to provide treatment strategies of this polymicrobial infection. Our group aimed to address this lack of information, specifically the effect of co-incubation on *C. albicans* in polymicrobial biofilms through a transcriptomic approach.

Monomicrobial and polymicrobial biofilms of *Candida albicans* and *Pseudomonas aeruginosa* were formed *in vitro*. RNA was extracted and prepared for sequencing. RNAseq was accomplished on the NextSeq500 and a relevant bioinformatics pipeline was utilized to determine differentially expressed genes of *C. albicans*. The resultant data was evaluated in two ways. Firstly, overrepresented Gene Ontology (GO) terms were determined with PANTHER. Secondly PathoYeastract was utilized to determine overrepresented transcription factors. The combination of these would allow an overview of affected processes in *C. albicans* in response to *P. aeruginosa.*

A large amount of *C. albicans* genes were differentially expressed in response to co-cultivation with *P. aeruginosa* (2537 open reading frames) corresponding to overall metabolic alterations and morphological changes. In addition, *SET3* and *WOR1* were differentially expressed, previously not attributed to the interaction with *P. aeruginosa*.

This study identifies a facet of interaction not previously described that could affect the virulence of *C. albicans* during co-infection with *P. aeruginosa* and dynamics of infection.

Keywords: *Candida albicans*, Interaction, *Pseudomonas aeruginosa*, Transcriptomics

Characterization of Wild *Saccharomyces cerevisiae* Strains Isolated from Fermented Foods to Select New Probiotic Yeasts

POSTER PRESENTATION ID: 134

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Yeasts exhibit numerous and diverse biological activities that make them promising candidates for a wide range of biotechnology applications. In addition to their major contribution in food industry being widely used as starters in the production of various foods and beverages, yeasts can possess antagonistic activities toward undesirable microorganisms, being considered as novel probiotic organisms. Strains from different yeast species are considered probiotic, mainly *Saccharomyces cerevisiae* var. *boulardii* and *S. cerevisiae* (1, 2). A strain of *S. cerevisiae* var. *boulardii* has also been recommended for the prevention and treatment of several types of gastroenteritis in children and adults (3). Nowadays, probiotic yeasts can be delivered either in fermented foods or as lyophilized cultures administered orally. This work was focused on the study of the probiotic potential of wild *Saccharomyces cerevisiae* strains in order to be used as integrator for human health as well as functional starter cultures for fermented products.

Fifty-two indigenous yeasts, isolated from fermented foods, were tested *in vitro* for probiotic traits, such as resistance to gastrointestinal conditions, simulation of the transit into gut, tolerance to bile salts, cell surface hydrophobicity ability, antioxidant activity (4,5).

The results showed a high variability among the strains for the majority of the tested parameters. In particular, three strains exhibited the closest traits to probiotic activity, due to their high resistance to body temperature, high survival at low pH, resistance to bile salts, as well as the ability to tolerate high concentrations of different antibiotics. Moreover, these selected strains showed some interesting probiotic traits in terms of simulation of the transit into the gut, hydrophobicity of the cells surface, high content of glucans and high antioxidant activity.

Further studies are in progress for the evaluation of these selected strains through *in vivo* experiments to promote them as new probiotic yeasts.

Keywords: Probiotic, Wild yeast selection

Production of γ-aminobutyric Acid in Dairy *Kluyveromyces marxianus* Strains

POSTER PRESENTATION ID: 139

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γ-aminobutyric acid (GABA) is a four carbon non-protein amino acid that is widely distributed in plants, animals and microorganisms with beneficial effects on human health (e.g. hypotensive, tranquilizing, diuretic, antidiabetic and blood-pressure-lowering effects in mild hypertensives). The ability to produce GABA has been reported in different yeast species such as *Wickerhamomyces anomalus*, *Saccharomyces cerevisiae*, and *Debaryomyces hansenii* among others. In the present study, 50 *Kluyveromyces marxianus* strains were tested for GABA production. Moreover, new primer sets were developed to study the expression of genes involved in GABA production and degradation.

The nucleotide sequences of *GAD1*, *UGA1* and *UGA2* genes were aligned with a Muscle alignment, using the software Mega, and displayed phylogenetically using a Neighbor-Joining method bootstrapped to 1000. On the basis of single nucleotide polymorphisms detected in *GAD*1 gene, 10 strains were selected and tested for GABA and succinic acid production by HPLC. qRT-PCR was performed with novel species-specific primer sets to evaluate the expression of *GAD1*, *UGA1* and *UGA2* genes.

On the basis of *GAD1* gene SNPs it was possible to split the group of 50 strains into 2 separate groups. Five strains from each group were selected and tested for GABA production. All selected strains were able to produce GABA with quantities ranging from 2.33±0.42 mg/L to 7.78±1.67 mg/L. *GAD1* gene was not upregulated in 2 strains while for the others fold changes ranged from 1.4±0.09 to 4.1±0.31. Under the conditions tested a very low concentration of succinic acid was detected and *UGA1* and *UGA2* were not upregulated.

This study represents the first study about GABA production on a population of *K. marxianus*. The variability observed confirmed the genetic and phenotypic biodiversity of this species. Further studies are necessary to establish the optimized conditions for GABA production. These strains could be used as starter cultures to develop fermented health-oriented products.

Keywords: γ-aminobutyric acid, *Kluyveromyces marxianus*, *GAD1*, *UGA1*, *UGA2* genes

Genome Sequencing and Characterization of Halophilic Yeast *Debaryomyces* spp. Isolated from Korean Traditional Fermented Foods

POSTER PRESENTATION ID: 164

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Fermented soybean products have been getting the spotlight in the international market due to their nutritive value and many health benefits. During soybean fermentation, yeasts play salient roles in the production of diverse flavor compounds that are important to the quality of the soybean products. In this study, we analyzed the genome structure, flavor profiles, and immune modulation activity of halophilic yeast *Debaryomyces* spp. KD2 and C0-11-Y2, which were isolated from Korean traditional fermented soybean products, called "Jang".

PacBio Sequel system with Illumina Hi-seq technology were used for whole genome sequencing. RNA-Seq was performed by Illumina TruSeq technology. Flavor profiles were analysed by SPME-GC/MS. Immune modulation activity was evaluated by analysing surface markers and cytokine levels of human dendritic cells using flow cytometry.

Debaryomyces hansenii KD2 and C0-11-Y2 strains, isolated from "Jang", showed much higher halotolerance than *Saccharomyces cerevisiae*. Moreover, they grew better in the presence of salts, indicating they are halophilic. The ploidy analysis by FACS and whole genome sequencing revealed that the genome of KD2 strain is haploid with the size of approx. 13 Mb, whereas that of C0-11-Y2 strain appeared to be diploid with the size of 26 Mb. They produced flavors of butter, caramel, and cheese in common, but the two strains exhibited different flavor profiles in the SPME-GC/MS analysis. Notably, the *Debaryomyces* spp. induced higher level of IL-10, an anti-inflammatory cytokine, than the established probiotic yeast *Saccharomyces boulardii*.

The *Debaryomyces* spp. isolated from "Jang", particularly *D. hansenii* KD2, shows its high potential as yeast starter cultures with enhanced halotolerance, fine flavour, and as novel candidates for probiotic yeasts to improve and globalize Korean traditional Jang products with high quality and functionalities.

Keywords: *Debaryomyces*, Halotolerance, Jang, Whole genome sequencing, Flavor, Immune modulation

Acknowledgement: This project was financially supported by the National Research Foundation of Korea, Grant No. NRF-2017M3C1B5019295 (Steam Research Project) and by the Korean Ministry of Agriculture, Food, and Rural Affairs, Grant No. 918010042HD030 (Syrategic Initative for Microbiomes in Agriculture and Food).

Biodiversity of Yeasts from Collected Honey

POSTER PRESENTATION ID: 176

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Thailand has the advantage of diverse natural resources which high biodiversity of life as well as microorganisms including yeasts. Bees are often present in completely nature also produce honey which is rich in nutrients that have many health benefits. Sugars in honey are also a source of food for microbes. Among them, they have high potential to grow in places that are not suitable for growth, such as high stress environment, low humidity, high concentrations of sugar. We hypothesized that yeasts found in honey may have special characters; therefore, we studied the biodiversity of microorganisms in honey bees. Different genetic identification and biochemical properties were evaluated.

Yeast strains isolated from raw honey bee samples were classified by PCR-amplification genus and species specific. Identification isolated strains were tested by PCR-amplified intergenic transcribed spacer region sequencing of rDNA (ITS1-5.8S rDNA-ITS2). All isolated strains were tested a specific SNP present in *SDH1* gene by PCR-amplification.

The results showed that 7 yeast strains isolated from raw honey bee were classified as 4 *Saccharomyces cerevisiae*. Other 3 isolated strains were tested by PCR-amplified ITS assays, which can be used for accurate molecular identification of yeast and microorganisms found that one strain was *Torulaspora pretoriensis* (identity 99.76%), one strain was closely related (identity 93%) to *Candida riodocensis*, and another had the closest similarity (identity 99.18%) to *Candida etchellsii*. Moreover, these isolated strains and some control strains were test for a specific SNP present in *SDH1* gene as well as contributes to the production of acetic acid and it is present in all *S. cerevisiae* var. *boulardii* strains investigated. However, none of the tested strains had these specific mutations.

A mix population of microorganisms from raw honey bee samples was found. Further characterization of yeast strains will be carried out for improvement of robust yeasts for applications in biochemical production.

Keywords: Wild yeast, Raw honey, SNP, *SDH1*, Acetic acid

Acknowledgement: Biodiversity-Based Economy Development Office (Public Organization), National research council of Thailand, king Mongkut's university of technology thonburi.

Bioadditives with Possible Probiotic Action, Obtained from Substrates Coming from Agroindustrial Waste Fermented with Yeasts

POSTER PRESENTATION ID: 178

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The use of additives such as: biocatalysts, enzymes, essential oils, bioactive compounds and efficient microorganisms (bacteria and yeast) is currently increasing in livestock industries, due to the its ability to improve intestinal health, decrease the concentration of pathogens and regenerate atrophied microvilli improving the absorption of the main nutrients, among others; on the other hand, they shorten the polymers present in food in smaller molecules, increase the degradation and digestion of nutrients and reduce ruminal methanogenisis. However, the availability and cost of these compounds limit their use. Nevertheless, the use of agroindustrial waste could be efficient and economical to develop bioadditives with probiotic action. Therefore, the aim of the study was to evaluate the biological additive obtained from a substrate coming from the residues of the agroindustry (molasses-vinasses) fermented with yeast.

50 litters of base substrate obtained from vinasses-molasses was used. Two types of microorganisms were used: *Saccharomyces cerevisiae* and *Kluyveromyces fragilis*. Biomass is obtained by separately inoculating 20 mg ($8.5x10^8$ CFU) of strains in 500 ml of sterile skimmed milk. The study variants were: T1, substrate + biomass with *S. cerevisiae*; T2, substrate + biomass with *K. fragilis* and T3, substrate + biomass with two yeasts. All substrates were incubated for 36 hours at 30 °C. According to a completely randomized design, a total of three treatments with six repetitions each were developed. In order to be candidates for probiotics, in the bioadditives obtained, physical-chemical characteristics, microbiological and *in vitro* tests (tolerance to pH <3.5, gastric juices, bile salts, growth inhibition of *E. coli* and *Salmonella* spp.) were evaluated.

At the beginning, all the treatments showed a dark brown colour, a sweet aroma and a pH lower than 4.28, specifically, this parameter, after 72 hours post-obtaining was established at 3.75, no significant differences were observed between variants (P> 0.05). The T1, T2 and T3 chemical composition was: 18.33; 18.51 and 22.63% of crude protein (P<0.0031); 12.40, 12.38 and 14.50% of true protein (P<0.0011), respectively, whereas, parameters such as: ethereal extract, ash and lactic acid concentration did not show significant differences (P> 0.05). The viability and microbial concentration were higher than 95% and $9.5x10^9$ CFU/mL in T3 (P<0.0021 and P<0.0010), respectively. *In vitro* tests: in T3, pH differed to P<0.0023, whereas, bile salts, gastric juices and the inhibitory effect of *E. coli* and *Salmonella* spp. did not differ (P<0.05) between treatments.

The bioadditives obtained from molasses-vinasses fermented with yeasts demonstrated physical-chemical and microbiological properties suitable for bioproducts. The results of *in vitro* tests evidenced the probiotic potential for veterinary purposes.

Keywords: *Kluyveromyces fragilis*, Molasses, *Saccharomyces cerevisiae*, Yinasses

An Evaluation of Probiotic Saccharomyces Strains Against Foodborne Pathogens

POSTER PRESENTATION ID: 232

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Although the most commonly used microorganisms as probiotics are the lactic acid bacteria, yeasts (e.g. *Saccharomyces cerevisiae*) and spore formers are also widely used in probiotic preparations. *S. cerevisiae* strains are especially preferred to probiotic bacterial strains due to availability of opportunistic pathogenic species among the bacterial probiotics. In the face of the rising antimicrobial resistance all around the world, probiotics have become a new source of treatment to fight with foodborne pathogens. This study aims to investigate scientific literature via a systematic review approach in order to highlight the newest information on the effect of probitic *S. cerevisiae* strains on foodborne pathogens including their mechanisms of action.

According to the PRISMA-P checklist (Moher *et al.* 2015), a protocol was developed detailing the specific objectives, the criteria for eligibility and study selection, and the reporting results. A comprehensive literature search was undertaken using PubMed, Web of Science, Cochrane Central, Medline and Scopus databases from their inception to September 20, 2019. Language was not restricted. Additional strategies to identify studies included manual review of reference lists of key articles that fulfilled our eligibility criteria, use of the "related articles" feature in PubMed, use of the "cited by" tool in Web of Science and Google Scholar, and manual review of reference lists of key articles. After dublicates and articles excluded based on abstact, a full-text assessment was conducted for the remaining articles. Mix formulations were discarded from the evaluation.

According to the 18 studied included in this analysis, results demonstrate that majority of the studies (77.8%) were conducted with public funding and in most of the studies commercial S. boulardii strains were used against the foodborne pathogens. The percentage of the yeast strains isolated from food origin was only 22.2% whereas the 16.7% of the studies did not report the origin. Among these 18 studies, 12 reported in-vitro, 3 reported in-vivo, and 3 reported in-vivo & in-vitro experimental designs. Regarding the mechanism of action, 2 studies found no effect on the pathogenic strain(s), 2 reported exclusion, 2 reported immunomodulation, 2 reported direct antagonism, 2 reported immunomodulation & exclusion and 1 reported direct antagonism & exclusion. The foodborne pathogens included in these studies were found to be: *Escherichia coli* (mostly EHEC strains), *Salmonella enterica* (mostly serovar Typhimurium), *Staphylococcus aureus, Campylobacter jejuni, Aspergillus flavus, Clostridium botulinum,* and *Yersinia enterocolitica.*

Keywords: *Saccharomyces*, Probiotics

Biotechnological Production of Polyols Through Conversions of Crude Glycerol by Newly Isolated Strains of the Yeast *Yarrowia lipolytica*

POSTER PRESENTATION ID: 244

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The purpose of this study is to investigate the ability of newly isolated *Yarrowia lipolytica* strains to grow on crude glycerol, the main by-product of the industrial production of biodiesel. In particular, the ability of the yeasts to metabolize glycerol and produce microbial mass and secondary metabolites such as cellular lipid, endopolysaccharides and polyols (e.g. mannitol, arabitol, erythritol) was assessed, in trials performed under nitrogen limitation. Two newly isolated strains (namely FMCC Y-74 and FMCC Y-75) were used, while trials were performed in different pH values (3.0-7.0), different initial glycerol concentrations (initial glycerol, $Glol_0$, varying between 40 and 120 g L^{-1}) and a fed-batch fermentation in shake flasks.

At low $Glol_0$ concentrations (40 g L^{-1}), almost exclusively mannitol was synthesized, while the more the pH dropped the more increment of polyols occurred (i.e. the strain FMCC Y-74 produced 20 g L^{-1} of mannitol at pH=3.0). On the other hand, when $Glol_0$ concentration increased (up to 80 or 120 g L^{-1}), besides mannitol, also arabitol and erythritol were synthesized in appreciable quantities. In fed-batch fermentations in which glycerol was maintained in substantially low concentrations (≤40 g L^{-1}), almost exclusively mannitol was synthesized (maximum mannitol *c.* 38 g L^{-1}, conversion yield of mannitol produced per unit of glycerol consumed 0.56 g g^{-1}). Cellular lipids in restricted quantities (8-14% in DCW) were produced, but in any case, lipid in slightly higher quantities were found at the first growth steps decreasing at the stationary growth phase, while cellular polysaccharides increased with the time reaching to values of *c*. 35-42% w/w in DCW at the stationary phase of growth.

Keywords: *Yarrowia lipolytica*

Acknowledgement: The current investigation was financially supported by the project entitled "Adding value to biodiesel-derived crude glycerol with the use of Chemical and Microbial Technology" (Acronym: Addvalue2glycerol, project code T1ΕΔΚ-03002) financed by the Ministry of National Education and Religious Affairs, Greece (project action: "Investigate – Create – Innovate 2014-2020, Intervention II").

Yeast Genetic and Genomic

Identification of Dolichol Kinase Mutations Responsible for Enhanced Secretion of Recombinant Proteins in *Kluyveromyces lactis*

POSTER PRESENTATION ID: 105

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The demand of secreted recombinant proteins rapidly increases. Nevertheless, yields of secreted recombinant proteins in yeast usually is far from optimal and needs optimization. *K. lactis* is extensively used as a model organism in the fundamental studies and biotechnology industry for production of metabolites and recombinant proteins including secreted proteins. The aim of this study was identification and characterization of gene and its mutations conferring the enhanced secretion phenotype of *K. lactis* mutant stain MD2/1-9.

The mutated gene was identified after sequencing of genomes of both parental and mutated *K. lactis* strains using the next-generation sequencing technology. For introduction of mutations into *K. lactis* genome CRISPR-Cas9 technology was applied.

Alignment of the sequenced *K. lactis* genome sequences revealed that mutation in ORF5776 might be responsible for the super-secretion phenotype in MD2/1-9 strain. ORF5776 encodes protein 43% identical to dolichol kinase (DK) of *S. cerevisiae* which is encoded by *SEC59* gene. Based on this similarity, we concluded that the mutated protein in *K. lactis* is likely DK, which catalyzes CTP-dependent phosphorylation of dolichol, and named the gene *KlSEC59*. After reversion of G/A substitution in the DK gene sequence, which resulted in a G405S amino acid change in *K. lactis* MD2/1-9 mutant strain, back to G, the level of secretion of α-amylase used as a marker for secretion was restored to the level of parental MD2/1 strain. The introduction of this mutation into another *K. lactis* strain CBS2369 revealed that for enhanced secretion besides G405S additional mutation I419S was needed. I419S mutation is one of three mismatches found in DK sequences of different *K. lactis* strains according Genbank databases. Other two, H34N and E164D, mismatches were not important for DK activity.

In summary, we found that compared with the temperature-sensitive *S. cerevisiae sec59-1* mutant, which exhibits reduced N-glycosylation and decreased secretory efficacy, the identified *K. lactis* DK mutations had less effect on DK activity, protein glycosylation deficiency, cell wall integrity and survival of yeast cells at high temperature. Moreover, despite some glycosylation defects, the double mutant strain with G405S and I419S mutations in DK sequence displayed improved secretion of recombinant *B. amyloliquefaciens* α-amylase, *S. cerevisiae* Kex2 proteinase lacking the transmembrane domain and human growth hormone fused with α-factor signal sequence relative to WT cells.

Keywords: *Kluyveromyces lactis*, Secretion, Glycosylation, Dolichol kinase, CRISPR-Cas9

Regulation of the Expression of a Methanol-Induced Transcription Factor Mpp1 in the Methylotrophic Yeast *Candida boidinii*

POSTER PRESENTATION ID: 125

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Methylotrophic yeasts have strong methanol-inducible gene promoters and have been used as hosts for heterologous protein production systems. To achieve efficient protein production by methylotrophic yeasts, it is important to elucidate the molecular basis of methanol-inducible gene expression in these yeasts. We have identified and characterized several transcription factors (Trm1, Trm2, Mpp1, and Hap complex) responsible for methanol-inducible gene expression in the methylotrophic yeast *Candida boidinii* (1-5). Among these transcription factors, Mpp1 was strongly induced by methanol. In this study, we analysed the regulation of the expression of *MPP1* gene in *C. boidinii*.

Promoter-reporter assay revealed that the *MPP1* promoter activity was absent in the *trm1Δ*, *trm1Δtrm2Δ*, and *hap3Δ* strains. But the *MPP1* promoter activity in the *mpp1Δ* strain was almost the same level as that in the wild-type strain. To determine the DNA sequences that are responsible for methanol induction in the *MPP1* promoter, a series of deletion promoters was constructed and the reporter enzyme activity was determined. As a result, we found three regions which showed drastic loss of the reporter activity.

The methanol-induced expression of the *MPP1* gene was depended on other transcription factors, Trm1, Trm2, and Hap complex, but not on Mpp1 itself. Further promoter deletion analysis revealed that the *MPP1* promoter has at least three methanol response elements. A proposed molecular mechanism for transcriptional regulation of methanol-induced genes will be discussed.

Keywords: Methylotrophic yeast, Gene expression

Examination of the Inheritance of mtDNA and Nuclear DNA in Three-Species Interspecific *Saccharomyces* Hybrids Produced by Mass-mating

POSTER PRESENTATION ID: 127

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Interspecific yeast hybrids (usually chimeras) isolated from nature frequently possess genomic parts from *more* than two species. We created three-species hybrids artificially by mass mating. First we crossed a *S. uvarum* (a, ade- ura-) haploid auxotrophic strain with a *S. kudriavzevii* (α, ura- trp-) haploid auxotrophic strain. Then we crossed the ura- allodiploid (kudvarum) hybrid with a leu- haploid *S. cerevisiae* strain. Then we selected prototrophic (presumably allotriploid; cekudvarum) hybrid colonies on minimal medium. The allotriploidy of the prototrophic hybrids was verified by karyotyping and PCR/RFLP analysis of chromosome-specific markers.

We checked the spore forming capability of the trihybrid strains. All of them formed spores well, but the spores did not form colonies. Sterility barrier came into action. We proved the presence of parental chromosome sets in the di-, and trihybrid strains by pulsed field gel electrophoresis. It was clearly demonstrated that the *S. uvarum* and the *S. kudriavzevii* chromosomes were all present in the two-species *kudvarum* hybrids. But because it is not easy to recognize the third partner's chromosomes, we labelled a *S. cerevisiae Y'* telomere probe with DIG-dUTP and then detected the *S. cerevisiae* chromosomes in the *cekudvarum* karyotypes with Southern blotting. The hybridization signals demonstrated that the trihybrid strains possessed also chromosomes having *S. cerevisiae* ends. So the *cekudvarum* trihybrid strains have got the genomes of all three parental strains.

We examined molecular markers by PCR/RFLP on the sixteen chromosomes of the partners: with some exceptions the *S. kudvarum* hybrids had both parental alleles, the *S. cekudvarum* hybrids had usually all three parental alleles of the genes. The RFLP analysis of the mtDNA revealed both parental and recombinant mitochondrial genomes in the *S. kudvarum* hybrids. But the *S. cekudvarum* strains usually got only the *S. cerevisiae* mtDNA. Only a few had recombinant-type patterns. The results of the PCR/RFLP analysis of the *COX2*, *COX3* and *ATP6* genes and the *COX1* introns correlated with the whole-mtDNA RFLP patterns.

Key words: Interspecies, Hybrid, *Saccharomyces*, Alloploid, mtDNA

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Genomic and Transcriptomic Analysis of *Candida intermedia* Reveals Genes for Utilization of Biotechnologically Important Carbon Sources

POSTER PRESENTATION ID: 147

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A future biobased society relies on efficient industrial microorganisms that can convert all sugars from agricultural, forestry and industrial waste streams into fuels, chemicals and materials. To be able to tailor-make such potent cell factories, we need a far better understanding of the proteins responsible for the assimilation of biotechnologically important carbon sources including pentoses, disaccharides and oligomers. The yeast *Candida intermedia*, known for its superior growth on xylose owing to its efficient uptake and conversion systems, can also utilize a range of other important carbon sources such as cellobiose, galactose and lactose. The aim of this project was to identify the genomic determinants for the utilization of these mono- and disaccharides in our in-house isolated *C. intermedia* strain CBS 141442.

Genome sequencing and transcriptional (RNA seq) data analysis during growth in defined medium supplemented with glucose, xylose, galactose, lactose or cellobiose, revealed numerous distinct clusters of co-regulated genes. By scanning the CBS 141442 genome for genes encoding Major Facilitator Superfamily (MFS) sugar transporters, and the RNA-seq dataset for the corresponding transcriptional profiles, we identified several novel genes encoding putative xylose transporters and multiple Lac12-like transporters likely involved in the uptake of disaccharides in *C. intermedia*. We also found that the yeast possesses no less than three genes encoding aldose reductases with different transcriptional profiles, and heterologous expression of the genes in *Saccharomyces cerevisiae* showed that the aldose reductases have different substrate and co-factor specificities, suggesting diverse physiological roles.

Taken together, the results of this study provide insights into the mechanisms underlying carbohydrate metabolism in *C. intermedia*, and reveals several genes with potential future applications in cell factory development.

Keywords: Carbohydrate metabolism

Mechanism of Adaptation to High Temperature Stress in Super Thermotolerant *Saccharomyces cerevisiae* SPY3

POSTER PRESENTATION ID: 152

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Thermotolerance of the yeast *Saccharomyces cerevisiae* is one of the important characters to reduce the production cost of bioethanol since it contributes to decreasing the cooling cost in fermentation in tropical countries where biomass energy is much abundant. A wild *S. cerevisiae* strain SPY3 isolated in Philippines was found to show thermotolerance up to at 42°C and displayed excellent ethanol production under high temperature conditions. Although it is the best thermotolerant wild *S. cerevisiae* as far as we know, its superior thermotolerant mechanism is not clear. To elucidate the mechanism, transcriptomic and molecular genetic analyses of SPY3 were performed in this study.

Microarray analysis was performed with a thermosensitive lab strain BY4743 and SPY3. Total RNA was extracted from these cells and genes expressed 2-times higher or lower in SPY3 than in BY4743 were elucidated and classified by their functions. Expression of up-regulated genes was analyzed by real-time PCR under 40°C condition and effect of the gene disruption and overexpression on the thermotolerance was analyzed. Accumulation of intracellular reactive oxygen species (ROS) was also analyzed using H2DCFDA as a ROS probe under 40°C condition.

Microarray analysis revealed that 419 genes were up-regulated and 362 genes were down-regulated in SPY3. After functional classification of these genes, antioxidant genes were found to be up-regulated in SPY3. ROS are reported to be generated under high temperature conditions and cause damage to cell. Real-time PCR analysis showed that antioxidant genes involved in ROS removal were highly expressed more than 10 times in SPY3 compared to those in BY4743 at 40°C. In fact, intracellular ROS level increased in BY4743 in response to high temperature stress of 40°C. However, SPY3 showed a much lower increase in ROS level at 40°C, indicating that SPY3 has a superior ability to induce antioxidant genes and reduce ROS level. Transcriptional activators Ace2, Sfp1 and Gcn4 were found to be potentially involved in the induction of more than 60% of the up-regulated genes in SPY3. Indeed, these genes were highly expressed in SPY3 and disruption experiments revealed that Ace2, Sfp1 and Gcn4 played important roles in superior thermotolerance of SPY3. These results suggest that functions of these transcriptional activators such as cell separation, ribosome biogenesis and amino acid biosynthesis contribute to improving thermotolerance of yeast.

Keywords: *Saccharomyces cerevisiae*, Thermotolerance

Acknowledgements: This work was supported by JSPS KAKENHI Grant Number 19K05790.

Metabolic Engineering of Lignocellulosic Yeast to Co-Ferment Cellobiose and Xylose

POSTER PRESENTATION ID: 165

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Lignocellulosic biomass is a promising renewable feedstock for bioethanol production that does not compete with food and animal feed[1]. However, the use of this biomass as a renewable alternative to fossil fuels requires efficient and sustainable break-down of its polysaccharides- cellulose and hemicelluloses to fermentable sugars- glucose and pentose sugars xylose and arabinose respectively, and their subsequent utilisation is crucial for the cost-effective and sustainable production of chemicals and fuels. The industrial workhorse for ethanol production, *Saccharomyces cerevisiae*, cannot utilise non-hexose sugars[2]. Genetically modified *S. cerevisiae* can ferment glucose and xylose; however, a significant bottleneck in achieving efficient and cost-effective co-fermentation of mixed sugars is the yeast's preferential uptake of glucose that delays the uptake of non-glucose sugars[3]. Recent studies demonstrated that the partial hydrolysis of cellulose to cellobiose instead of glucose, subsequent uptake and intracellular hydrolysis of cellobiose is an option for the alleviation of glucose repression by an engineered *S. cerevisiae* expressing a cellobiose transporter and an intracellular beta-glucosidase[4,5]. Moreover, this enables the co-fermentation of cellobiose and other non-glucose sugars. The industrial yeast strains are robust, but their genetic manipulations are limited by the availability of selection markers and genome-editing tools. In this study, we aim to engineer an industrial, xylose-fermenting strain of *S. cerevisiae* also to ferment cellobiose via a combinatorial genome-editing method. Our genome-editing approach involves the advanced genome-editing tool CRISPR-Cas9 for marker-free multi-copy gene integration in the yeast genome. The cellobiose uptake and intracellular hydrolysis will be optimised by creating a library of transformants with a range of different gene copy numbers, followed by a competitive cultivation scheme to isolate clones with high and fine-tuned levels of gene expression.

Keywords: Cellobiose, Lignocellulose, Bioethanol

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Chromosome Doubling of Allodiploid Yeast by Hydrostatic Pressure

POSTER PRESENTATION ID: 170

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There is currently a great interest in interspecific hybridization of *Saccharomyces* yeasts to produce strains with novel characteristics. Allodiploid strains resulting from interspecific hybridization are usually sterile because homeologous chromosomes fail to pair. Therefore, it is difficult to make hybrids using allodiploid strains as parents. Colchicine, however, is known to induce chromosome doubling in plant species and has been used to restore fertility of interspecific hybrids. Furthermore, hydrostatic pressure has been reported to induce chromosome doubling of *Saccharomyces cerevisiae* and *Schizosaccharomyces pombe*. Here, we present the results of our investigation whether hydrostatic pressure confers mating ability to allodiploid yeast strains through chromosome doubling.

Hydrostatic pressure was given to an interspecific hybrid of *S. cerevisiae* and *S. eubayanus* resulting in the appearance of strains with chromosome doubling. The frequency of diploidization was 2% to 8%. Despite the fact that parental allodiploid strains cannot sporulate, asci of the chromosome doubling strains were observed after induction of sporulation. Spores of these asci could form hybrids with *MAT*a or *MAT*α type laboratory strains, indicating that these spores have the ability to mate.

Our results suggest that chromosome doubling induced by hydrostatic pressure is an effective means to confer mating ability to allodiploid yeast strains.

Keywords: Interspecific hybridization, Mating ability, Hydrostatic pressure, Chromosome doubling, Allodiploid

Acknowledgements: We would like to thank Dr. Chiaki Kato of Japan Agency for Marine-Earth Science and Technology (Present; Nonprofit organization Team Kujira-Go) for the scientific advice.

Linking Essential Genes to Yeast Stress Tolerance Phenotypes Using CRISPR Interference Technology

POSTER PRESENTATION ID: 183

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Saccharomyces cerevisiae is extensively used as a model organism and to link genotypes to phenotypes. Several large-scale screenings of various mutant collections have been performed in different growth conditions. Screening the yeast deletion library has revealed many genes important for specific growth conditions. A major drawback of this library is nonetheless that it does not include any essential genes as the deletions of those result in lethality. In this study, we are screening a yeast library consisting of >9000 strains, where the expression of one of the 1,117 essential or 514 respiratory growth-related genes can be regulated using the CRISPR interference (CRISPRi) technology.

The CRISPRi technology is a genetic perturbation technique that allows sequence-specific repression of gene expression, achieved by a catalytically inactive Cas9 protein fused to a Mxi1 repressor, which can be targeted to any genetic loci using an sgRNA. Depending on the targeting location of the sgRNA, genetic repression ranging from very strong to weak can be achieved. We have been performing high-throughput phenotyping of the CRISPRi library under weak acid stress using the scan-o-matic high resolution screening platform.

We found several novel genes that when repressed can lead to a change in tolerance towards weak acid stress. Weak acid stress tolerance is a highly desirable phenotype for industrial production hosts as the presence of inhibitory weak acids in second-generation-biomass raw materials is a severe bottleneck in the production of biochemicals. Therefore, the results of the high-throughput phenotyping screens will allow us to design new bioengineering strategies for obtaining improved yeast strains for second-generation biochemical production.

Keywords: CRISPR-interference, Stress regulation, Weak-acid tolerance, Lignocellulose, Yeast strain bioengineering

Yeast DNA Barcode Database a Tool for the Repository for *S. cerevisiae* and Other Fungi

POSTER PRESENTATION ID: 185

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The identification and classification of *S. cerevisiae* and other yeast species commonly used in food and biotechnology is considered to be an important research topic. For this purpose, 120 isolations were obtained from different sources (commercial cultures, collection cultures, universities research cultures, Central Anatolian grape and soil samples), classical definitions, MALDI-TOF definitions and molecular definitions are done on these isolations. After the definitions of the isolations we searched barcode structures using different gene regions (ITS, LSU and RPB2). All research information obtained from the isolations is recorded in a database ("www.dnabarcodefoodomics.com"). The web-based registration system works on two approaches; identification queries and registration information forms of isolation. As a result, it is thought that the creation and recording of DNA barcode information and thus providing quick access to the information in this field has contributed to the competitiveness for our country which is an important international yeast producer. Obtained barcode gene regions and barcode sequences can be searched by this system according to species.

Keywords: DNA barcoding, *S. cerevisiae*, Yeast

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Genetic Bases of Nitrogen Requirement in Wine Yeast Assessed Through QTL Analysis

POSTER PRESENTATION ID: 221

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In grape must, nitrogen content is often insufficient for the completion of alcoholic fermentation by yeast. For *Saccharomyces cerevisiae*, response to nitrogen deficiency is strain-dependent, some strains being able to complete fermentation despite nitrogen deficiency whereas others are not and result in sluggish or stuck fermentation. Thus, it is of high interest to study the mechanisms behind those different responses and exploit them to improve yeast strain for wine fermentation when nitrogen content is low. Previous study highlighted different genomic regions involved in nitrogen requirement through BSA (*Bulk Segregant Analysis*), and the contributions of three genes: *MDS3, GCN1, and ARG81* have been shown (1). However, many other large genomic regions were also defined for which we could not find evident candidate genes. In addition, BSA did not provide any information on possible interactions between loci.

In order to explore further the genetic bases of nitrogen requirement, we applied a QTL analysis to the fermentation rate in nitrogen deficient medium, on a population of 131 individually genotyped segregants obtained from the same cross as (1). The dense genetic map available for the segregant population (3727 markers) enabled us to perform single and multiple map QTL and thus define genomic regions which could be implied in low nitrogen requirement. In order to further validate the impact of candidate genes on the phenotype, alleles were "swapped" by CRISPR-*Cas9* technique and phenotype was evaluated in comparison with haploid parent strains.

Several regions with high LOD scores were identified, some above the significance threshold, and others below, among which the regions containing the genes identified by (1), probably in relation with the multigenic character of the trait. In the region with the highest LOD score, two candidate genes in relation with nitrogen metabolism (namely, Target of Rapamycin (TOR) pathway and lifespan regulation) were identified. In addition, in order to reveal possible interaction between genes, strains carrying different combinations of *GCN1* and *MDS3* parental alleles (implied in TOR pathway) have been evaluated. These constructions confirm their role on the fermentation rate in low-nitrogen conditions and indicate dependence on the genetic background.

These results confirm the complexity of mechanisms involved in nitrogen requirement during alcoholic fermentation and will permit to optimise wine yeast strain selection in response to winemaking industry demands.

Keywords: Nitrogen requirement, Wine, Yeast, TOR, QTL

Transcriptome Analysis of the *Saccharomyces cerevisiae* Cells Reveals Early and Late Events in Their Response to High Concentration of Ethanol

POSTER PRESENTATION ID: 228

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Yeast *Saccharomyces cerevisiae* is a very common model in life sciences research as well as an industrial organism. In the industrial context yeast cells are exposed to various potentially toxic compounds and stresses, including the main product of fermentation, ethanol. Knowledge on the mechanisms of ethanol tolerance by yeast cells is crucial for their successful employment in the industry. High ethanol affects yeast cells in multiple ways so it is assumed that it generates a combination of other stresses, such as cell wall and plasma membrane integrity, protein misfolding, hyperosmotic and oxidative stresses. Therefore the multifaceted cell protection mechanisms are to be expected. Indeed, this multitude of stress effects is reflected in the results of transcriptome analyses of the yeast cell response to short term exposure to high levels of ethanol. They consistently show the induction of hundreds of genes functionally related to various stresses, indicating that short term exposure to high ethanol triggers mostly the response to environmental stress. However, during the industrial fermentation process yeast cells are exposed to high ethanol concentration for a long time and our knowledge on their long-term response to these conditions is limited.

Our time-course transcriptome analysis of *S. cerevisiae* cells response to high ethanol revealed distinct functional groups of genes up-regulated at various time points after cells exposure to these conditions. In addition to rapid and transient response to stresses provoked by high ethanol there is a late, sustained response to the presence of this compound. The functions of these genes indicate their involvement in yeast cell adaptation to changed environment.

This late response is likely to be important for long term survival of yeast cells under fermentation conditions. Revealing the genes conferring tolerance to high concentration of ethanol and determining the mechanisms of their regulation expands our knowledge on the processes involved in ethanol adaptation. This will provide the theoretical, scientific background for more precise selection of genome modifications leading to improvement of performance of *S. cerevisiae* strains in the industrial milieu.

Keywords: Transcriptome analysis, Regulation of gene expression, Fermentation, Ethanol stress

Defects in Vesicular Trafficking Leads to Loss of Genome Integrity

POSTER PRESENTATION ID: 231

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The implementation of genome-wide studies to the yeast biology research allowed to learn more cellular mechanisms considered as significant for the genome maintenance. In our previous studies, by using three functional genomic screens (sensitivity to zeocin, sensitivity to overexpression of endonucleases and ploidy shift) we found vesicular trafficking as a process that contributes to maintenance of the genome.

Among 63 strains lacking various vesicular trafficking genes indicated in the screens several strains displayed highly increased DNA fragmentation in comet assay. These strains showed more phenotypes linked to genome instability such as increased sensitivity to genotoxic stresses, lowered recruitment of Rad52-YFP to the DNA lesions, which suggests impaired DNA double strand breaks recognition or DNA repair defects. The frequency of spontaneous Rfa1-YFP foci formation was increased in comparison to control strain and did not increased in response to genotoxic stress. Moreover, these strains displayed the DNA content alterations in flow cytometry analysis.

Accumulated data indicate the moon lighting role of vesicular trafficking proteins in cellular response to DNA damage.

Keywords: Genome stability, DNA repair, Stress response, Vesicular trafficking

Acknowledgements: This work was supported by NCN grant 2016/21/B/NZ3/03641

Whole-Genome Transformation Allows for Rapid Improvement of Selectable Traits Through Temporary Protection by a Functional Genetic Element of a Tolerant Strain

POSTER PRESENTATION ID: 250

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Yeast cell factories are capable of producing an array of chemicals and pharmaceuticals in a more environmentally friendly manner. However, the conversion of agricultural and municipal waste to valuable compounds by these yeast biorefineries is currently economically less favourable than the chemically synthesized fossil fuel-based alternatives. A solution would be to implement a consolidated bioprocessing approach where yeast secretes lignocellulolytic enzymes that break down plant biomass and ferments the released carbohydrates to the desired product. A major hurdle here is the significant difference between the optimal fermentation temperature of *Saccharomyces cerevisiae* (±35°C) and the optimal hydrolytic temperature of the enzymes (45°C-55°C). Improving yeast thermotolerance could therefore be a suitable approach to reduce bio-compound production costs and facilitate the switch to a bio-based economy.

Whole-genome transformation was used to improve high-temperature fermentation performance of a haploid segregant of an industrial *Saccharomyces cerevisiae* strain through transformation with gDNA of thermotolerant yeast species *Kluyveromyces marxianus* or *Ogataea (Hansenula) polymorpha*.

Whereas the parent strain, ER18A, could only ferment 40-50% of the maximal theoretical yield at 42°C, the transformants readily fermented all sugar in about 24 hours. Three transformants were submitted to whole-genome sequencing and a small number of putative SNPs were identified, not originating from either of the donor species. CRISPR/Cas9-mediated allele exchange in the transformants revealed the involvement of the essential *TRT2*-encoded $tRNA^{Thr}_{CGU}$. In transformants KEA17 and KEA24 an anticodon change in a $tRNA^{Lys}_{CUU}$ and $tRNA^{Met}_{CAU}$, respectively, to that of the essential $tRNA^{Thr}_{CGU}$ resulted in fully functional mutant tRNAs capable of taking over the function of $tRNA^{Thr}_{CGU}$. These data also suggest that the threonyl-tRNA synthetase can aminoacylate these mutant tRNAs and therefore appears to use the anticodon as main structural recognition element. The same anticodon changes as happened in KEA24 apparently happened during evolution in *Xenopus tropicalis* and six other species. Many more of such anticodon switching events appear to have occurred throughout evolution in all kingdoms.

Whole-genome transformation is a rapid and easy method to improve selectable phenotypes in yeast. None of the mutations identified in the transformants are originating from the donor gDNA. Altogether, our data suggest that the gDNA of a tolerant strain could have a temporary protective function until the yeast generates novel SNPs and/or other genomic modifications. Analysis of the superior transformants points towards an important role for *TRT2* in early heat stress adaptation and highlights the plasticity of the yeast tRNA landscape.

Keywords: Whole-genome transformation, Thermotolerance, tRNA

Yeast General

Crude Glycerol and Hemicellulose Hydrolysate as Feedstock for Microbial Biodiesel Production

POSTER PRESENTATION ID: 149

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Crude glycerol is a by-product of biodiesel transesterification, and the produced amounts have led to global abundance of crude glycerol. In this project this currently very cheap carbon source was tested as a possible substrate for oleaginous yeasts for lipid production. Series of screening were performed to identify strains capable to utilise crude glycerol and hemicellulose hydrolysate as carbon source.

Screenings on 27 strains of oleaginous yeast species were performed on plates using the sterile disk method, droplet test and in shake flask cultivations. Based on analysis of carbon source utilisation using HPLC, a strain of red yeasts was selected as it had highest growth rate and glycerol consumption: *Rhodotorula toruloides* CBS 14. It was subsequently grown in single batch setups. The first batch medium contained 40% hemicelluloses hydrolysate mixed with 60 g/L crude glycerol, the second batch medium contained only 60 g/L crude glycerol. The *R. toruloides* CBS 14 was additionally tested in two types of media: (I) 10% hemicellulose hydrolysate mixed with 50 g/L crude glycerol and (II) 50 g/L crude glycerol.

The highest measured average biomass and lipid content were achieved in the first batch cultivation of *R. toruloides* - 19.4 g/L and 10.6 ± 0.3 g/L with a lipid yield of 0.224 g lipids per consumed g of carbon source (22.4%). The first batch also had the highest volumetric glycerol consumption rate of 0.44 g/h. Fatty acid composition was similar to other *R. toruloides* strains. Most dominant was oleic acid (C18:1, up to 45.95%), followed by palmitic acid (C16:0, up to 28.2%), linoleic acid (C18:2, up to 16.2%), stearic acid (C18:0, up to 12.5%) and α-linolenic acid (C18:3, up to 3.2%).

All of these fatty acids are commonly found in vegetable oils used for biodiesel production leading to conclusion that crude glycerol enriched with hemicellulose hydrolysate using *R. toruloides* CBS 14 has a good potential as carbon source to produce biodiesel.

Keywords: *Rhodotorula toruloides*, Oleaginous yeast, Crude glycerol, Hemicellulose, Biofuels

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In vitro and in vivo assay of Epigallocatechin Gallate Effect on Yeast Prion Protein Sup35p Aggregation

POSTER PRESENTATION ID: 214

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Protein aggregation into amyloid fibrils is related to neurodegenerative diseases, such as Alzheimer‘s, Parkinson ‘s and prion diseases. Compounds that could inhibit such aggregation and prevent the onset or progression of these diseases can become potential drugs. A polyphenol from green tea known as epigallocatechin-3-gallate (EGCG) was shown to inhibit amyloid fibril formation of different proteins and peptides, but the inhibition is not fully associated with EGCG autoxidation and its products. The objective of this research was to determine whether EGCG derivatives can inhibit the formation of amyloid fibrils by yeast protein Sup35p, both *in vitro* and in *Saccharomyces cerevisiae*.

EGCG autoxidation was followed by fluorescence maxima shift. Thioflavin T fluorescence assay was used to follow amyloid formation of recombinant Sup35p NM domain (Sup35NM) *in vitro*. Imaging of fibrils was done using AFM. Amyloid formation *in vivo* was observed using *S. cerevisiae* 74D-694 [psi-][PIN+] strain. Cells were cultivated in synthetic (SC) medium.

We found that EGCG derivatives clearly slows down the amyloid aggregation of Sup35NM *in vitro*. ThT fluorescence value decreases upon the increase of inhibitor concentration. Also, autoxidized EGCG postpones the formation of [PSI+] amyloid fibrils in *S. cerevisiae* by 20 hours. Moreover, EGCG does not inhibit yeast cell growth when growth media contains all necessary additives.

These findings suggest that EGCG autoxidation is necessary to develop an inhibition of aggregation. EGCG derivatives interact with Sup35p *in vitro* slowing the elongation of aggregation nucleus. Furthermore, *in vivo* findings show weaker inhibition effect than examined *in vitro*.

Keywords: Sup35NM, EGCG

The Effects of Chemical and Physical Agents on Elimination of [PSI] Prion Protein Aggregates in *Saccharomyces cerevisiae*

POSTER PRESENTATION ID: 217

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Yeasts *Saccharomyces cerevisiae* has been widely used in genetics and cell biology. They are serving as a model for all eukaryotes, including humans, for the study of fundamental cellular processes. One of the processes which are actively studied is the protein folding mechanism. Protein misfolding is a common event in cells which is handled by eliminating such proteins. However, sometimes cells are not capable to eliminate all misfolded proteins which lead to protein accumulation, assembly into aggregates, and cell death. Yeast has several proteins that are self-propagating amyloids of normally soluble proteins and because of that, they are infectious. These proteins are called prions. The objective of this research was to find chemicals and physical agents that eliminate existing [PSI] prion protein aggregates in *S. cerevisiae* cells.

S. cerevisiae 74D-694 strain cells with different [PSI] prion variants were cultivated with chemical compounds: glycerol (0–30 %), KCl (0–2.5 M), and Guanidine HCl (0–7 mM). *S. cerevisiae* 74D-694 strain cells were affected with physical agents: pulsed electric field (PEF) and heat-shock (42 °C). YPD and SC yeast cultivation media were used.

In this study, we found a significant effect of glycerol on [PSI] prion protein elimination in *S. cerevisiae* yeast cells. After 72 hours of cultivation with glycerol, around 7 % cells became [PSI] prion protein aggregates free. GuHCl as a traditional cure of prion protein in yeast showed similar treatment efficiency: from 7 to 10 %. Furthermore, the use of glycerol and GuHCl together with PEF, and heat-shock showed synergistic effects. No significant effect of KCl on [PSI] prion protein elimination was found in *S. cerevisiae* yeast cells.

This study revealed glycerol as a new chemical compound that eliminates the yeast [PSI] prion protein aggregates. It is possible that glycerol eliminates prion protein aggregates by causing osmotic stress on cells, but further research is needed to determine the exact way it works.

Keywords: [PSI] prion, Guanidine HCl

Cloning and Enhanced Expression of the Recombinant Mussel Foot Adhesive Protein-1 in *Pichia pastoris*

POSTER PRESENTATION ID: 252

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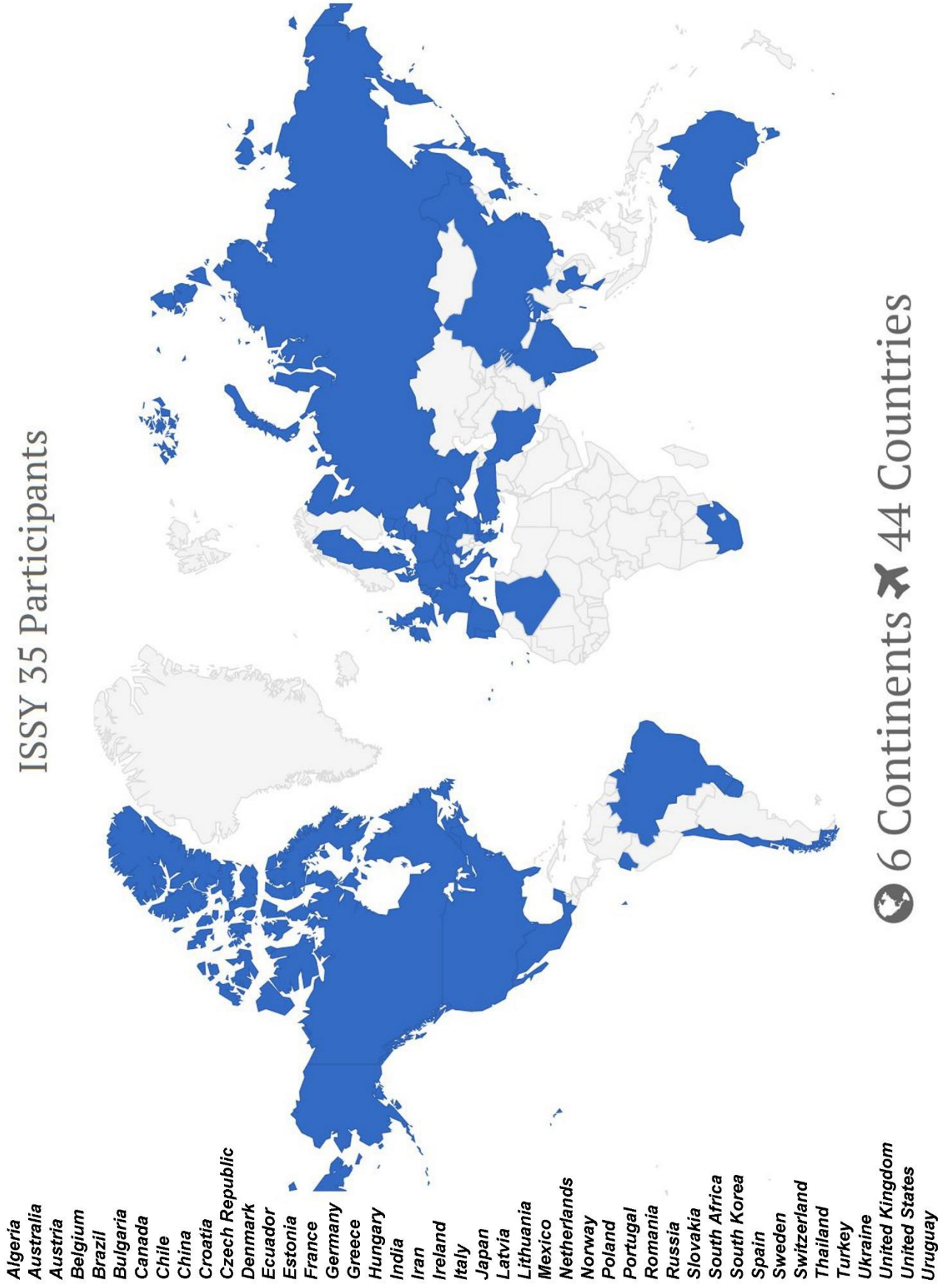
Mussel foot proteins (Mfps) have been suggested as one of the alternative bioadhesives for biomedical application mainly due to their ability to work in wet condition with less immunogenic reaction in human body. However, many studies have been failed to produce functional and feasinle recombinant Mfps in several hosts for clinical aapplications due to the low efficiency.

In the present study, we cloned and successfully expressed Mfp1 in *Pichia pastoris*. For this purpose, mussel foot protein 1 (Mfp1) sequence was obtained from NCBI databases with the size of 1680 bp and codon optimization was performed for expression in *P. pastoris* as the host using the shuttle vector pPiczalphaA. Linearized vector then transformed into the yeast by electroporation. Polymerase chain reaction was used to confirm the insertion of the target sequence with Mfp1 specific primers. Alcohol oxidase promoter before Mfp1 sequence could be induced by methanol. We checked the different concentrations of methanol to obtain the best yield.

Here, we report the presence of Mfp1 in the supernatant after the induction procedure by western blotting using anti-HIS antibody, which was designed at the C-terminal. Colony PCR results showed that the linearized vector successfully integrated into *Pichia pastoris* genome. Using a BMM medium with 0.5 percent methanol resulted in the highest yield. SDS-PAGE and western blotting results also confirmed the presence of the expressed protein in the supernatant with a size of 67 kDa.Various genetical and biochemical approaches were applied to enhance its expression followed by purification and characterization.

Our data suggested that *Pichia pastoris* could be used as a suitable host for the expression of Mfp1. Besides considering glycosylation property of the expressed protein it will be more compatible with clinical application compared to the bacterial expression systems.

Keywords: Mfp1, Bioadhesive protein, *P. pastoris*, Expression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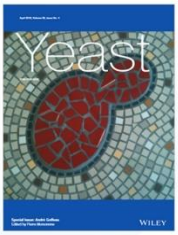

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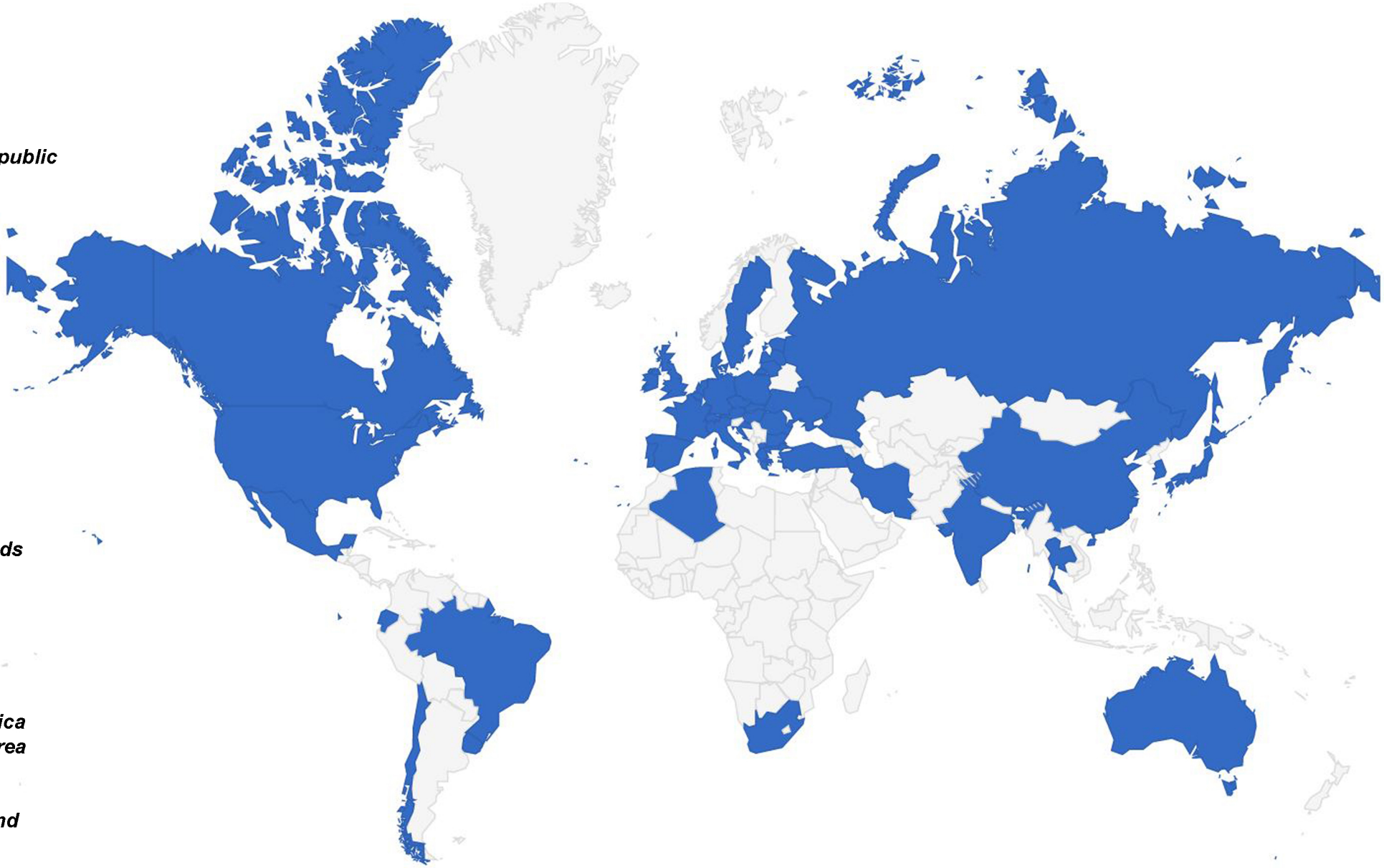

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